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THE EFFECT OF SINGLE AND MULTIPLE DOSES OF THE PARATHYROID HORMONE ON THE CALCIFICATION OF THE DENTIN OF THE RAT INCISOR*

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INTRODUCTION

The specific effect of the parathyroid hormone on calcium metabolism has, with few exceptions, been generally accepted. The dentin of the rat incisor has been found to be extremely sensitive to changes in calcium metabolism. Erdheim,[1] in his parathyroid autotransplant experiments on rats, demonstrated a calcium-free stripe of dentin that corresponded in position and width with the time and duration of the interval that elapsed between the removal of the parathyroid and the "taking" of the transplant. Schour and Ham [2] studied the effect of single doses of ergosterol and parathyroid hormone on the dentin of the rat incisor. They were able to correlate definite histological reactions with the blood calcium findings and, particularly, with the periods of rise and fall of the blood calcium. It was, therefore, found advisable to investigate further the effect of single and multiple injections of varying doses of parathyroid hormone on the calcified tissues of the rat incisor, particularly the dentin, and to correlate these effects with the blood calcium changes and the alterations in other tissues in the body which have been fully described in a previous report.[3]

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TABLE I

Data on 29 Rats that Were Given Single and Multiple Injections of Parathyroid Hormone, Arranged in Order of Number of Injections[1]

Rat No.	Weight	Duration of experiments	Dosage units and times injected	Time between last injection and death	Blood serum calcium	Necrosis of kidney	Histological changes in the dental tissues					
							Enamel	Dentin			Alveolar bone	
							Enamel hypoplasia	Calcification		Vascular or cellular inclusions	Predominance of osteoclasts	Fibrous marrow spaces
								Poor immediately after first injection	Excessive subsequent to first injection			
	gm.	*days*		*hrs.*	*mg. per 100 cc.*							
316	91	1	50 (1X)	19	13.0	–	–	+	–	–	–	–
317	107	1	75 (1X)	19	13.6	+	–	+	–	–	–	–
318	118	1	100 (1X)	19	12.6	+	–	+	–	–	–	–
320	86	1	100 (1X)	19	9.2	–	–	+	–	–	–	–
374	125	2	150 (1X)L	48		++	–	+	+	–		
376	80	3	140 (1X)	72	11.2		–	–	–	–	–	–
380	125	2	150 (1X)L	48	14.8	++	–	–	–	–	–	–
300	125	2	45 (2X)	19			–	Slight	Slight	–	–	–
336	130	3	25 (3X)L	19	13.7	–	–	Slight	Slight	–	Slight	–
337	143	3	25 (3X)L	19	11.2	–	–	–	Slight	–	Slight	–
338	135	3	25 (3X)L	19	12.4	–	–	+	+	–	Slight	–
322	118	3	50 (3X)L	19	17.1		–	+	+	–	Slight	–

TABLE I — *Continued*

331	122	10	50 (3X)L	19	12.1	++	−	+	+	−	Slight	−
332	110	3	50 (3X)L	19	15.2	++	+	+	+	+	+	−
333	120	3	50 (3X)L	19	14.4	++	+	+	+	−	+	−
334	125	3	50 (3X)L	19	15.5	++	−	+	+	−	+	−
370	120	3	50 (3X)*	19	10.8		−	−	−	−	−	−
371	105	3	50 (3X)*	19	10.7		−	−	−	−	−	−
301	104	6	15 (4X)	72	13.0	+	+	+	+	+	−	−
302	118	5	22 (4X)	19	14.8	+	−	+	+	−	Slight	−
303	147	4	30 (4X)	19			−	+	+	−	−	−
324	130	7	50 (4X)	19	17.0	++	+	+	+	+	+	+
323	146	10	50 (9X)	19			+	+	+	+	+	+
326	145	15	10 (12X)	19	11.4	−	−	−	−	−	−	−
327	130	15	10 (12X)	19	10.8	−	−	−	−	−	−	−
307	88	16	18 and 36 on alternate days (12X)	19	13.7	++	−	+	+	+	−	−
308	117	13	23 and 46 on alternate days (13X)	19			−	+	+	−	Slight	−
306	85	18	17 and 34 on alternate days (14X)	19	15.4	++	+	+	+	−	+	+
305	115	15	**(15X)	19	22.0		−	+	+	−	+	−

L = parathormone

* = inactivated hormone

** = this animal received 17 to 23 units for the first 3 days, the following period from 23 to 46 units, and finally 24 hours before being killed 75 units. A total of 450 units was given. The animal was very sick the day following the last injection.

[323]

[1] The following bracket figures represent the numbers of the animals and their table in the preceding report [2]: 301 (1, T. 3), 302 (2, T. 3), 306 (3, T. 3), 316 (5, T. 3), 317 (4, T. 3), 318 (6, T. 3), 320 (7, T. 3), 322 (1, T. 2), 324 (2, T. 2), 326 (3, T. 2), 327 (4, T. 2), 331 (5, T. 2), 332 (6, T. 2), 333 (7, T. 2), 334 (8, T. 2), 336 (9, T. 2), 337 (10, T. 2), 338 (11, T. 2), 370 (12, T. 2), 371 (13, T. 2), 374 (19, T. 3), 380 (20, T. 3).

Method and Material

This study is based on the same material that McJunkin, Tweedy and Breuhaus [3] used in their investigation of the effects of the parathyroid hormone on the parathyroids and on the tissues of the rat.

The series consisted of 29 rats weighing from 80 to 147 gm. The doses of parathyroid hormone varied from 1 to 15 in number, and from 10 to 150 units in quantity. In animals that were given repeated doses the interval between the injections varied from 24 to 72 hours (Table I). The animals were killed usually 19 hours after the last injection. Seven controls from the same colony and within the same age limits as the experimental animals were studied histologically. In addition the histological data of the dental tissues of 104 normal rats used in other studies conducted by the senior author were available.

Histological Methods: The heads of the animals were fixed in 10 per cent formalin immediately after death. A midsagittal section of each head was made to facilitate X-raying. The teeth and their investing tissues were then dissected further in order to facilitate fixation and histological preparation. The tissues were washed, decalcified in 5 per cent nitric acid, treated with sodium sulphate, washed, dehydrated and embedded in celloidin. The sections were cut and mounted in serial order and stained with hematoxylin and eosin. A small percentage of the sections was also stained with iron hematoxylin and Mallory's connective tissue stain. Midsagittal and transverse sections were prepared in each case. In a number of animals ground sections of the hard tissues alone, and ground sections of both the hard and soft tissues, stained with hematoxylin and eosin or with alizarin, were also prepared.

Chemical Methods: Before the animals were killed blood was drawn by cardiac puncture and the calcium analysis made, in most instances, on a 1 cc. sample of serum. The Kramer-Tisdall method, as modified by Tweedy and Koch, was employed.

The blood serum calcium of 7 rats picked at random from the same colony and on the same stock diet ranged from 10.62 to 11.60 mg. per cent. The diet consisted of fox chow (commercial preparation) supplemented by lettuce, cheese and lean meat.

The hormone preparation designed "L" in the tables is parathormone (Eli Lilly and Company). The preparation not so designated was prepared by the method of one of us (Tweedy [4]) and standardized by Collip and Clark's procedure.[5] The acid alcohol inactivated hormone preparation is described by Tweedy and Torigoe [6] in a separate publication.

Histophysiology of Dentin of Normal Rat Incisor

Dentin, like bone, consists of an organic matrix substance which becomes calcified. It is apposed at the pulpal surface in the form of layers, which in the adult rat grow forward at the same rate as that of eruption. The position and width of a given layer indicate the time it was formed and its duration.

The dentin matrix is calcified in the form of globules that are normally small and numerous and so close together that there results

a uniformly calcified tissue. But even in the normal dentin the successive layers of dentin are not equally well calcified. Well calcified layers alternate more or less regularly and rhythmically with imperfectly calcified layers, so that there arises a stratification in the dentin, especially toward the incisal edge (Schour [7]). There is a distinct smooth boundary between the matrix that was laid down last, called predentin, and the calcified dentin matrix (Fig. 1).

The staining reaction of dentin to hematoxylin and eosin is a dependable, although not a perfect, indicator for its state of calcification (Schour and Ham [8]). The dentin that takes on the hematoxylin homogeneously has been generally and, in the authors' opinion, correctly accepted as being well calcified. The dentin that does not take the hematoxylin stain has been generally regarded as uncalcified dentin and has been named predentin. The latter, as a rule, takes the eosin stain but, frequently enough as careful observation reveals, this layer is found to be entirely or in part devoid of the eosin stain also. There are, therefore, on the basis of the hematoxylin-eosin reaction, two types of predentin which, for convenience, will be named early and late predentin.

The early predentin is found next to the pulp and appears first. It reacts neither to the eosin nor to the hematoxylin stain and although it already contains dentinal tubules it reminds one of the membrana preformativa (Raschkow) of the dental papilla found in the tooth germ stage which just precedes the formation of the hard tissues. It is pale gray with the iron hematoxylin and pale blue with the Mallory connective tissue stain.

The late predentin takes the eosin stain. It is dark gray with the iron hematoxylin and dark blue with the Mallory connective tissue stain.

In given fields either stage may be absent. When both are present the late predentin is often found to intervene in the form of a narrow ribbon-like eosin stripe between the early stage and the calcified dentin. When only one stage is present it occupies the entire space between the pulp and the calcified dentin and the boundary between the latter and the particular stage is smooth and linear.

The specific staining of the dentin matrix of the rat incisor for argyrophil and collagenous fibers (Orban [9]) will show whether the early predentin represents a precollagenous matrix and the late predentin a collagenous one or not.

It is possible that the eosin-staining dentin is not calcium-free as has been generally supposed, but represents a preparatory stage of calcification. Whether the different staining reactions of the so-called predentin and the calcified dentin lie in a difference in the quantity of inorganic salts or in a difference in the combination of inorganic salts is not known.

Histopathological Findings

A few of the representative experimental animals will be described in detail.

Effect of Single Doses

The rats that were killed 24 hours after a single injection of 50, 75, or 100 units of parathyroid hormone, respectively, show an alteration in the calcification of the dentin formed during the experimental period (Table I). This disturbance is confined to the predentin layer which is slightly wider than normal (20 to 25 μ) and which consists of an eosin-staining portion that borders smoothly against the hematoxylin-staining dentin of the preoperative period, but irregularly against the portion that is adjacent to the pulp and that stains with neither hematoxylin nor eosin. This disturbance becomes aggravated with the increase in the number of units.

Figure 2, taken from Rat 317, which was given 75 units of parathyroid hormone, shows the eosin-staining predentin which is irregular where it faces the early or non-eosin-staining predentin. The latter, in addition, is not homogeneous but contains isolated eosin-staining globules.

Figure 3, taken from Rat 318, which was given 100 units, shows a more aggravated disturbance. The non-staining predentin is sprinkled with a greater number and a smaller size of eosin-staining globules. In addition, the dentin that was laid down just previous to the time of the injections takes a deep hematoxylin stain and is preceded by a lighter eosin-staining stripe.

Figure 5, taken from rat 374, which was given one dose of 150 units of parathyroid hormone, shows in the dentin that was formed during the 48 hours of the experimental period (about 30 to 35 μ) first an eosin-staining dentin and then pulpally a deep hematoxylin-staining stripe. The latter in some places is replaced and modified so that it does not react to hematoxylin or eosin. On the other

hand, Rats 376 and 380, which were also given single doses of 140 and 150 units respectively, showed no histological changes in the dentin.

Effect of Multiple Doses of Parathyroid Hormone

3 Injections of 25 Units Each (Rats 336, 337 and 338): The dentin situated within approximately 30 μ from the pulp takes a deeper hematoxylin stain (Fig. 4). There is, labial to this dentin, an indication of a lighter eosin stripe. Osteoclasts are seen more readily than normal in the labial alveolar bone.

3 Injections of 50 Units Each (Rats 322, 331 to 334, 370 and 371): The first 3 rats that were given 3 injections of 50 units each show a similar and typical reaction. The latter is characterized by an eosin-staining layer that corresponds with the dentin formed immediately following the first injection and a deep hematoxylin-staining layer that corresponds with the dentin laid down during the time of the 2nd and 3rd injections.

Some portions of the tooth, particularly the midregion in longitudinal sections and the lateral surfaces in transverse sections, show a disturbance and deviation from the typical reaction in respect to the staining reaction of the dentin hypercalcified during the period comprising the last 2 injections. In these regions the deep hematoxylin reaction may be absent entirely or in part, so that the corresponding dentin stains only with eosin or stains neither with eosin nor hematoxylin. The dentin formed within 24 hours of the time of death shows an eosin or non-staining reaction. The entire dentin involved extends from 35 to 46 μ.

In Rat 331, in which the experiment extended over a 10 day period, the reaction is similar but is found to be extended over a wider portion of the dentin and is less disturbed than in rats in which the same number of injections followed in closer succession. The boundary between the pulp and the predentin is wavy toward the anterior end of the pulp and the stratification is similar to that shown in Figure 9. Rats 370 and 371, which were injected with the inactivated parathyroid hormone preparation, showed a normal histological picture.

4 Injections of from 15 to 50 Units Each (Rats 301 to 303 and 324): Rat 301 will be taken as a representative of this group and the findings will be described in greater detail.

The enamel surface shows a region of severe enamel hypoplasia (Fig. 6). The latter is situated at approximately 1.6 mm. from Hertwig's sheath. The dentin next to this enamel defect is taking the eosin stain and marks the beginning of a stripe that can be traced toward the incisal edge (Fig. 6). The disturbance in the calcification of this stripe is least prominent in the posterior region (Fig. 7). Over the middle portion of the tooth this eosin stripe is 15 μ in width and is followed pulpally by a wider stripe of dentin, which stains deeply with hematoxylin (Fig. 8). In this figure the width of the dentin from the outer end of the eosin stripe to the pulp is 90 μ. This portion of the dentin represents the amount laid down during the duration of the experiment and approximates the width of dentin that would be laid down in a period of 6 days in a normal animal of similar age.

Thus the dentin laid down immediately following the first injection is poorly calcified and remains in this state. The dentin laid down subsequently is overcalcified and continues to be so throughout the remainder of the experimental period, except for the apparently normal predentin next to the pulp. The overcalcified stripe shows some variations. It sometimes takes a pale blue color next to the eosin stripe. In the posterior portion of the tooth its overcalcification is less intense (Fig. 7) than in the middle portion (Fig. 8). In the more anterior region it shows prominent stratification, and in addition vascularization (Fig. 9).

Rats 302 and 303 show similar pictures, except that the changes involve a smaller area of dentin (Fig. 12), since the experiments lasted only 5 days and the pattern of the reaction is severely disturbed in the midregion of the tooth. Figure 12 which is taken from the midregion shows a stripe that is quite pale blue in color. Rat 324, which had 4 injections, also showed a reaction that for the most part was similar to that observed in Rat 301. Vascular and cellular inclusions in the dentin are prominent (Fig. 15).

Multiple Injections of Parathyroid Hormone Greater than 5 in Number (Rats 305 to 308, 323, 326 and 327): Rat 323 shows changes similar to those of Rat 301. In addition, vascular and cellular inclusions in the dentin are more prominent. There is also prominent engorgement of the blood vessels in the pulp. The dentin in the molar shows a reaction in the form of a persisting eosin stripe which is followed by a deeper hematoxylin-staining dentin band and predentin (Fig. 14).

Rats 326 and 327, which were given 12 daily injections of 10 units, each show a normal histological picture. The stratification in the dentin is readily observed in the dentin nearer to the pulp but is within the range of normal variation.

Rat 307, which was also given 12 daily injections but of higher unitage (alternately 18 and 36 units), shows prominent and abnormal stratification like that seen in Figure 13, depicting the effect in Rat 306. The dentin shows vascularization in some places. Rat 308 shows some stratification which is irregular but, as a whole, normal.

Rat 306 shows prominent, irregular stratification consisting of eosin and hematoxylin-staining stripes with pale blue-staining bands interspersed. The area of stratification is 290 μ in width and approximates the amount of dentin that would be laid down in a normal animal of similar age in a period corresponding with the duration of the experiment (18 days). The dentin laid down previous to this width is normal (Fig. 13). There is severe enamel hypoplasia. The alveolar bone shows a predominance of osteoclasts and a fibrous change in the bone marrow (Fig. 11). Compare with Figure 10 which is taken from a corresponding section of control Rat 309. The dentin in the molars also shows an abnormal picture similar to that shown in Figure 14. Rat 307 shows a similar but less intense reaction than is seen in Rat 306.

The findings in the ground sections confirm those in the decalcified preparations.

Discussion

Effect of Single Injections: The layer of the predentin that is ready to be calcified at the time of the injection responds immediately to the experimental condition. For convenience this portion of the dentin will be called the primary hypocalcified stripe. It is characterized by its failure to stain with hematoxylin and by its reaction with eosin either in a homogeneous manner or, in the case of higher dosage (75 to 100 units), in the form of globules (Figs. 2 and 3). It is commonly accepted that an irregular boundary between calcified dentin and predentin in the form of globular projections is an indication of disturbed calcification. In the experimental condition of single high doses, also, we have a corresponding disturbance in the form of an irregular boundary, only the latter is situated between the late and early predentin. Apparently we are

dealing with a disturbance and retardation of the normal progressive transformation of early predentin into late predentin, and of the latter into the fully calcified dentin.

The findings in Rat 374, which was given an injection of 150 units and which was allowed to live 48 hours (Fig. 5), show that the primary injection stripe, which on the basis of its position belonged to approximately the first 24 postinjection hours, remained more or less unchanged. However, the dentin that corresponded to approximately the second 24 hour postinjection period, and which may be called the secondary hypercalcified stripe, showed a tendency to overcalcification. In this case of a single high dose the effect appears to be similar to that observed by Schour and Ham [2] in single 40 unit injections, but accelerated and more irregular. While they found overcalcification only during the approximately second 50 hour postinjection period, in Rat 374 this condition appeared during the second 24 hour postinjection period and showed in parts defective calcification as well. The dentin, thus, acted as if, following the high dose of 150 units, the blood calcium curve both reached its peak and declined twice as fast as was the case with the dosage of 40 units.

The left kidney was removed 48 hours before death from Rats 374, 376 and 380 for microscopic examination. The absence of response in the dentin in Rats 376 and 380 may be accounted for on the basis of differences in the degree of shock produced by the operative procedure.

Effects of 3 Injections: The reaction varies with the dosage and the duration of the experiment. The sequence of events in the rats that received 25 unit doses (Fig. 4) is similar to that seen following 50 unit doses, except that following the higher doses the overcalcification of the secondary stripe is somewhat irregular and lacks uniformity, and the primary stripe associated with the first effect of the first injection is more prominent.

Rat 331, in which the 50 unit injections were spread over a longer period of time (10 days), showed a reaction that was less intense than that of the animals of shorter experimental life and which, of course, extended over a wide width of dentin corresponding to the amount laid down in 10 days.

The absence of reaction in the rats that were given the inactivated parathyroid hormone preparation gives conclusive proof that the effects observed were due to the parathyroid hormone and not to

any foreign protein impurities. Further evidence was obtained from 2 rats that were given 2 injections of kidney substance and that showed a normal picture in their dentin. An extensive series of rats which were treated with other tissue extracts showed no reaction in the dentin (Schour [10]).

Effect of Multiple Injections (More than 3): The reaction follows the general pattern observed in the experiments with fewer injections, but shows more severe disturbances in the calcification of the dentin formed toward the latter part of the experiment. These alterations consist of vascular and cellular inclusions, irregular outline of the pulpal surface, and greater prominence in the interspersion of stripes which appear to have remained in the early predentin stage (Fig. 15).

Enamel Hypoplasia: The fact that in 5 rats enamel hypoplasia is found at the level where the primary injection stripe begins shows that the enamel defect started at the time of the first injection (Fig. 6). Apparently at that time the disturbances in calcium metabolism were so severe and intense that both enamel and dentin suffered an acute injury. The fact that the enamel hypoplasia began at the time of the first injection is confirmed in some rats by the measurement of the distance between the enamel hypoplasia and the Hertwig's sheath. The distance corresponds closely with the extent of the eruption of the teeth during the experimental period. With the exception of Rat 301, each rat that showed enamel hypoplasia also showed an abnormal increase in the number of osteoclasts. Since the enamel hypoplasia began as a rule at the time of the first injection and in no apparent correlation with the amount of the dosage, it is possible that the susceptibility of the individual rats played an important rôle in the reaction of the enamel and the enamel epithelium. The absence of enamel hypoplasia in the rats that were given single doses of large unitage may be explained on the basis of their brief postinjection life (19 to 48 hours). It is possible that these injections produced in the ganoblasts an immediate injury which, however, cannot be recognized histologically until the cells reach a more highly differentiated and functional state.

Summary of Dentin Reaction: In spite of considerable individual variations in respect to the intensity of the reaction the principal changes are similar. The dentin is disturbed in respect to the quantity and rhythm of calcification. Our present histological methods

are inadequate to point out to what extent the quality of calcification may be disturbed as well.

The extent and type of the disturbance varies not only with the amount and frequency of the injections, the duration of the experiment, the time interval between successive injections and between the last injection and the death of the animal, and to a limited extent with the individual animals, but also with the relative chronological position of the dentin in respect to the time of the injections. In practically all cases the dentin of the postinjection period shows: (*a*) the primary stripe which is characterized by incomplete and deficient calcification and which in its position corresponds with the time immediately following the first injection (see all figures except 10 and 11); and (*b*) the secondary stripe which is characterized by overcalcification and which corresponds in its position with the time subsequent to approximately the first 24 hour postinjection period (Figs. 5, 8 and 12), (Table I).

The findings in the "multiple injections" experiments show that the calcification of late predentin is more or less permanently disturbed, so that the primary injection stripe is constant and does not disappear.

The secondary stripe varies in width with the duration of the experiment, and in its pattern with the number and unitage of the doses. In the rats that were killed within 24 hours after a single injection the secondary stripe is naturally absent (Figs. 2 and 3), (Table I).

The primary stripe may be regarded as an immediate acute reaction to the first injection. The secondary hypercalcified stripe may be regarded as a chronic reaction and indicative of an effort at healing.

Both stripes vary, within limits, in their width and staining reaction with their position in respect to the anteroposterior extent of the tooth and in respect to their distance from the pulp. Any given reaction is found to be as a rule most prominent in the midportion of the incisor and least prominent in the more posterior portion, and appears to be reduced with the increasing distance from the pulp. The histological study was centered on the calcification of dentin. The possible changes in the odontoblasts have not been studied.

Theories on the Mechanism of Parathyroid Hormone Action: The mechanism by which there results a mobilization of calcium in the

blood following the administration of parathyroid hormone into a reactive animal has not been established. Three theories, however, are prominently in the foreground of the controversial literature on this subject. Briefly stated they are as follows. (1) The administration of parathyroid hormone makes the blood plasma a better solvent for the calcium compounds of the blood or other tissues. For example, Greenwald [11] was the first to suggest that there circulates in the plasma a substance "x," which is identical with parathyroid hormone, or is formed under its influence, and which unites with calcium ions to form an undissociated compound, thus reducing the concentration of calcium ions in the plasma and permitting the liberation of more calcium ions from bone. It is evident that this theory depends upon the conception that the plasma behaves as if in equilibrium with tricalcium phosphate. (2) The parathyroid hormone lowers the renal threshold for the inorganic phosphate, so that plasma phosphate decreases and the plasma calcium is enabled to rise. This view has been put forward by Albright and co-workers.[12] The basic idea of this theory is that the plasma inorganic phosphate and calcium bear a reciprocal relation to one another, and behave as if in equilibrium with tricalcium phosphate. (3) The parathyroid hormone directly stimulates the cellular elements in the bone to increased osteoblastic or osteoclastic activity. This view has been advanced by Selye.[13]

Space does not permit a critical discussion of the controversial literature dealing with these theories. The senior author and his associate in a recent paper [8] have discussed their reasons for believing that the experimental findings on which the last theory is based have been incorrectly interpreted. We also feel that the destructive critical evidence advanced by Thomson and Pugsley [14] casts grave doubts on the validity of the second theory. While we are not prepared to accept the first theory we feel that the experimental findings in this work fit in best with the conception that the parathyroid hormone controls a definite fraction of the plasma calcium.

Possible Basis for the Dental Changes in Experimental Hyperparathyroidism: We believe that, fundamentally, the pattern in the dentin represents its response to the changes in the calcium and phosphorous metabolism induced by the injections of the parathyroid hormone.

The fact that in single administrations of ergosterol and para-

thyroid hormone there were found, depending on the duration of experiments, both primary and secondary injection stripes, and the fact that these stripes were associated with a rise and fall of the blood calcium respectively, (Schour and Ham [2]) suggest a similar association in the experiments of this report. It is therefore possible that in each case there is at first a more or less sharp rise in blood calcium which results in the primary hypocalcified stripe, and subsequently a decline which results in the secondary hypercalcified stripe. It is also possible on the basis of Pugsley's work [15] that with 25 units of injections per day an increased excretion of calcium rather than a rise in blood calcium may be responsible for the presence of the primary hypocalcified stripe.

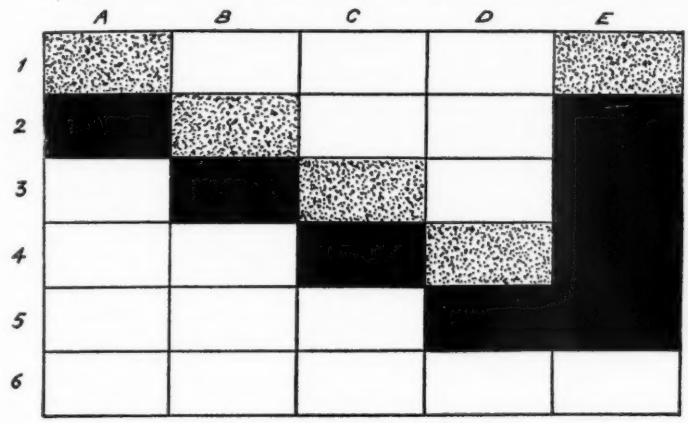
The association of a primary hypocalcified dentin stripe with hypercalcemia and of a secondary hypercalcified stripe with the return to a normal blood calcium value may be explained tentatively on the theory discussed in greater detail by Schour and Ham [8] that the parathyroid hormone controls a fraction of the serum calcium. Thus, an injection of the hormone results first in a shift of calcium from the tissues to the blood, possibly, to the fraction of the serum calcium controlled by the hormone either by calcium withdrawal or by the attraction of the calcium that is normally available for calcification, or both. With our present state of knowledge we are not in a position to state whether the hypocalcified condition of the primary dentin stripe was due to a calcium withdrawal or due to the fact that an insufficient amount of calcium was available when it was being calcified.

When the effect of the parathyroid hormone has passed the calcium that was held in solution under its influence is liberated and there results, therefore, a shift of calcium from the blood back to the tissues. Thus in the dentin that is being calcified at this time we find the secondary hypercalcified stripe, because a greater than normal amount of calcium is now available. It is also possible, according to Schour and Ham,[8] that the liberation of calcium from the blood might introduce more calcium into simple solution than could be retained without precipitation.

If in case of "multiple injection" experiments the injections were made at intervals sufficiently far apart so that the successive administrations were made only after the effect of the preceding ones had worn off, then we would have simply a series of pairs of primary

and secondary stripes, the number of pairs corresponding with the number of injections. We have a suggestion of such a reaction in Rat 306 (Fig. 13).

In the experiments in which the multiple injections were in daily succession only the primary stripe associated with the first injection remains relatively unchanged, while the rest of the stripes overlap and fuse into an extended secondary stripe which represents the summation of the effects of the successive injections (Figs. 6 and 8). It is likely that the blood calcium curve in such animals would show



TEXT-FIGURE 1

Diagram showing the histological effects of parathyroid hormone injections on the dentin. The horizontal sections represent the portions of dentin laid down in successive days, 1–6. The vertical columns, A–E, represent sections of dentin belonging to animals A–E respectively.

subsequent to the early rise a decline that was retarded by a plateau. In some of the experiments of multiple injections the histological findings suggest the presence of interruptions of the fall of blood calcium in the form of secondary rises associated with the repeated injections.

Text-figure 1 illustrates in diagrammatic form the histological effect of parathyroid hormone injections on the dentin. The findings in this report and that of Schour and Ham [2] indicate that if Rat A were given a single injection of parathyroid hormone at the beginning of the first day, Stripe 1 would be poorly calcified and Stripe 2 overcalcified, as indicated by column A, and shown in Text-figure 1;

similarly, if Rats B, C and D were given single doses on the second, third and fourth day respectively, the effects would be those indicated in columns B, C and D. It is obvious that if the above 4 injections were given on successive days to 1 Rat E instead of singly to 4, the effect would be that indicated in column E. The latter corresponds closely to the pattern seen in Figure 8 taken from a rat that was given 4 successive daily injections.

Blood Calcium Findings: Our blood calcium findings are of limited value because they indicate the condition immediately preceding death and therefore reveal only one point in the blood calcium curve of the experimental period. However, a comparison of the findings of animals that were given single injections and killed 19 hours later shows that the blood calcium rose in 4 of the 5 animals (Table I). Similarly a group of animals that were given 3 successive daily injections of 50 units each showed a prominent rise in blood calcium 19 hours after the last injection, while another animal with the same number and dose of injections, but distributed over a period of 10 days, indicated a return to normal at the time immediately before death.

Dentin Reaction in the Molar: The dentin in the molars appears to be normal in the majority of the experimental animals. In a few instances, however, the findings reveal an interesting picture. Figure 14 shows a prominent eosin-staining stripe, which is followed by a well calcified stripe that is next to an abnormally wide predentin border. The width of the region involved is approximately one-fourth that of the dentin region found to be disturbed in the incisor. If the molar dentin grew one-fourth the rate of the incisor dentin, one could assume that fundamentally the dentin reacts similarly in the incisor and molar. If the molar dentin grew much slower than one-fourth the rate of the incisor dentin one could assume the possibility of calcium withdrawal. The final explanation of the molar findings awaits further investigation.

Alveolar Bone in the Rat Incisor: Among the 21 rats that had 3 or more injections 14 showed an abnormally increased number of osteoclasts. The 7 rats that showed a normal alveolar bone include 2 that were given inactivated hormone and 2 that were given repeated doses of 10 units each. In 3 of the rats the alveolar bone shows a typical picture of osteitis fibrosa, in respect to the predominance of the osteoclasts and the fibrous change in the bone marrow (Fig. 11).

This is not at all surprising since the alveolar bone is highly active, especially at the age of the experimental animals. At this time the curvature of the incisor becomes flattened and the alveolar bone undergoes considerable adjustment to this change in contour. Bones that are most active have been found to be most susceptible to characteristic changes of experimental hyperparathyroidism (Jaffe [16]).

Differences in the Reaction of Dentin and Alveolar Bone to Experimental Hyperparathyroidism: Dentin records delicate changes that are not recognizable in bone and tolerates insults in calcium metabolism that produce severe disturbances in bone. Figure 13 shows the dentin disturbance in Rat 306 which was given 14 injections of parathyroid hormone. Each injection is recorded. The last 3 injections, which were given 48 hours apart, produced wider secondary hypercalcified stripes than did the earlier injections given 24 hours apart. But on the whole, the dentin is well calcified in spite of the disturbance in rhythm and is capable of normal function. On the other hand, the alveolar bone changes in the same rat, shown in Figure 11, are much more severe. The osteoblasts are replaced by osteoclasts. The bone is resorbed and liable to fracture. The bone marrow is fibrous. It appears that, in confirmation of Erdheim's calcioprotective law,[17] dentin possesses a greater tolerance against calcification disturbance than does bone.

On first glance there is an apparent contradiction between the high sensitivity and the high tolerance of dentin to changes in calcium metabolism. However, a consideration of the histophysiological processes of dentin formation and calcification shows that a given layer of dentin is found to be adjacent to a very rich blood supply only temporarily when it is being laid down in its organic form and when it is in the process of becoming calcified. At this stage it is very sensitive to changes in calcium metabolism. As new layers of dentin are laid down and the tooth is erupting forward a given calcified layer moves farther away from the blood supply and in its entirely avascular condition becomes less sensitive and more tolerant to disturbances in calcium metabolism. Moreover, the normal and slow process of secondary calcification may result in a recovery and improved calcification of a former primary hypocalcified stripe, seen in Figure 7.

The alveolar bone, on the other hand, while it is, as a rule, at no time exposed to as rich a blood supply as is found adjacent to the

newly formed dentin, is constantly penetrated by blood vessels and is in close proximity to them. It is, therefore, always exposed to changes in calcium metabolism disturbances. These considerations apply in a similar manner to other bones as well as to alveolar bone.

Variations in Calcification within the Same Incisor: The variations in calcification seen within the same tooth at various levels (Figs. 6, 7, 8 and 9) may be in part explained by corresponding variations in the proximity of blood supply at various levels of the tooth.

The blood supply of the pulp is richest in the posterior region and very poor in the anterior region. As a given stripe of dentin moves forward its posterior portion lies within the vicinity of a rich blood supply for a longer period than its anterior portion and is, therefore, subject to secondary calcification for a longer time. The anterior portion lies at the very outset within the vicinity of a poorer blood supply and moves farther away from it with the eruption of the tooth. Thus, the primary undercalcified stripe loses its prominence in its posterior portion (Fig. 7), and similar to the fate of the transplantation stripe of Erdheim [1] often shows greatest disturbance in the anterior zone (Fig. 9). There are, however, prominent variations seen within limited regions of the tooth which cannot be explained solely on the basis of blood supply. It seems that in the pathological disturbances induced in the experiments of this investigation the typical response itself suffers aberrations for reasons not yet known.

Sensitivity of Dentin: The dentin reaction in the guinea pig incisor has been employed successfully as an indicator for vitamin C deficiency. The delicacy of the dentin reaction to the various experimental conditions described in this report suggests the feasibility of using the dentin of the rat incisor as an indicator for a biological assay of the parathyroid hormone. The present common method of standardization of the parathyroid hormone by means of the blood calcium reaction in the dog is laborious and expensive. The authors believe that a simplified method would facilitate research on the parathyroid hormone. It is likely that further experimentation will obviate the necessity of histological preparation and evolve a simple method of preparing ground sections of the rat incisor which can be stained with alizarin or which can be obtained from rats given alizarin intravitally.

Summary and Conclusions

The effect of single and multiple injections of parathyroid hormone on the incisor of the white rat has been studied in 29 animals and 7 controls in respect to microscopic alterations and blood calcium changes.

The histological findings are: (1) enamel hypoplasia formation begins immediately after the time of the first injection in 5 animals; (2) the alveolar bone shows an abnormal increase in osteoclasts in 14 and a fibrous change of the bone marrow in 3 of the rats that were given 3 or more injections of parathyroid hormone; and (3) the principal changes are found in the calcification of the dentin. The experimental animals show a primary hypocalcified stripe in the dentin that was being calcified during the immediate effect of the first injection and a secondary hypercalcified stripe in the dentin that was being calcified subsequently. The extent of and the variations within the secondary stripe vary with the number and unitage of the hormone preparations injected, and also with the duration of the experiment.

It is believed that the experimental pattern in the dentin represents its response to the changes in the calcium and phosphorous metabolism induced by the injections of the parathyroid hormone. The dentin reaction can be explained best by the theory that the parathyroid hormone controls a fraction of the serum calcium.

Note: We wish to thank Eli Lilly and Company for the parathormone used in these studies, and Swift and Company for their coöperation. We also wish to thank Miss F. Schwab and Mr. H. C. Breuhaus for their assistance in the preparation of the material.

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DESCRIPTION OF PLATES

PLATE 89

FIG. 1. Photomicrograph of longitudinal section of midregion of upper incisor of a normal rat. Note the predentin layer, P, which stains neither with hematoxylin nor eosin. The eosin-staining late predentin is absent. D = normal calcified dentin; OD = odontoblasts. × 455.

FIG. 2. Photomicrograph of longitudinal section of midregion of upper incisor of Rat 317, which received one injection of 75 units of parathyroid hormone and was killed 19 hours later. Note the irregular boundary between the early, E, and late predentin, L, and the scattered eosin-staining globules in the early predentin. The secondary hypercalcified stripe is absent because the animal was killed too soon. D = dentin which is normal and which was calcified before the experiment began; OD = odontoblasts. × 427.

FIG. 3. Photomicrograph of longitudinal section of midregion of upper incisor of Rat 318 which received 1 injection of 100 units of parathyroid hormone. Note the poor, incomplete transition of early, E, into late predentin, L, and the scattered eosin-staining globules, GL, in the early predentin. Note the absence of the secondary hypercalcified stripe. OD = odontoblasts. × 427.

FIG. 4. Photomicrograph of transverse section of lower incisor of Rat 337, which was given 3 injections of 25 units each of parathyroid hormone. Both the primary hypocalcified stripe, PR, and the secondary hypercalcified, SE, are not prominent. The total width of both stripes corresponds with the amount of dentin that is laid down during the time of the duration of the experiment. Compare the staining reaction of the dentin formed before, D, and after the injections, PR and SE. OD = odontoblasts; PU = pulp. × 101.5.

FIG. 5. Photomicrograph of longitudinal section of midregion of upper incisor of Rat 374, which was given 1 injection of 150 units of parathyroid hormone and was allowed to live 48 hours. Note the primary hypocalcified, PR, and the secondary hypercalcified, SE, stripes. D = dentin calcified before the experiment began; OD = odontoblasts. × 210.

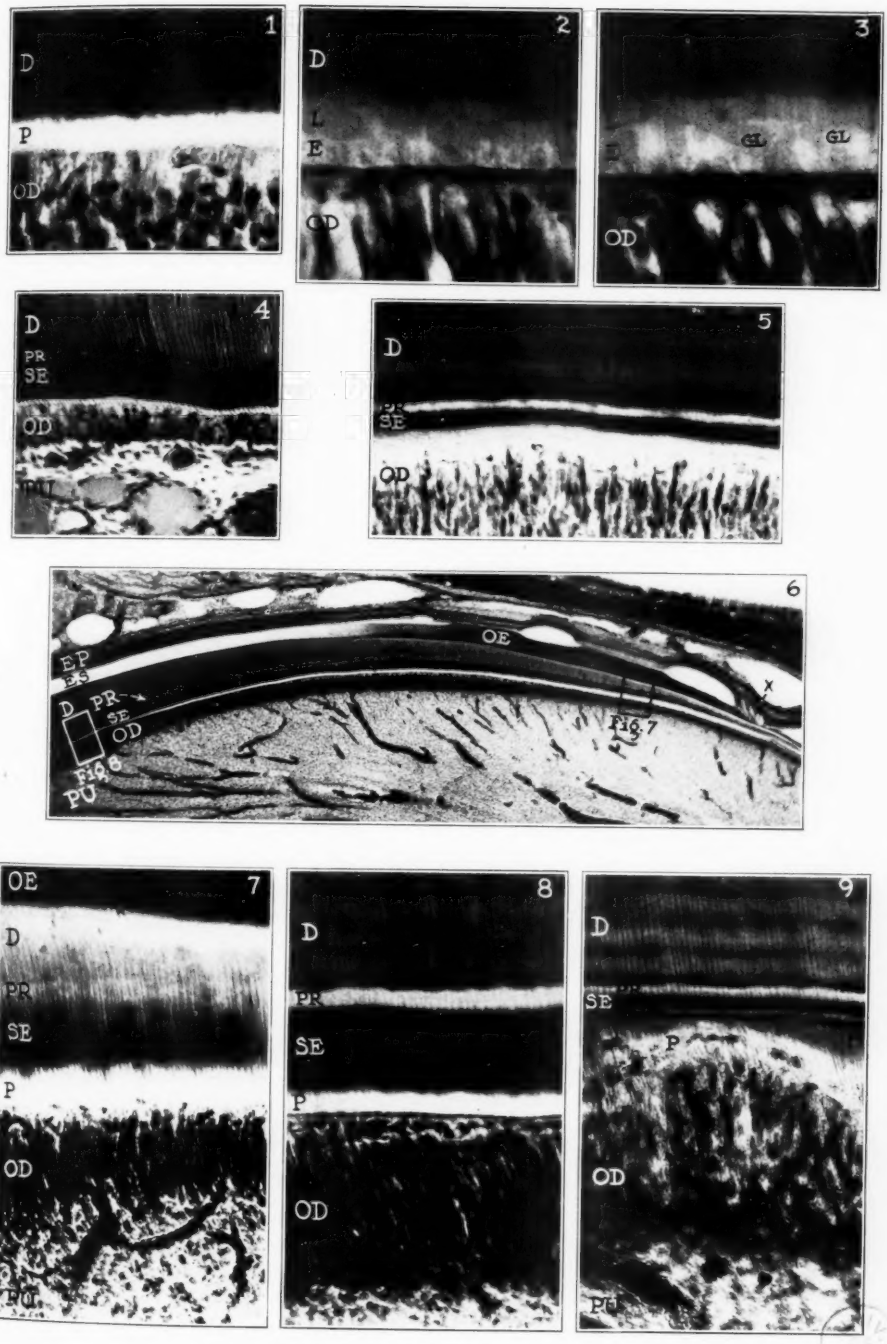
FIG. 6. Photomicrograph of longitudinal section of posterior and midregion of upper incisor of Rat 301, which was given 4 injections of parathyroid hormone and was allowed to live 3 days after the last injection. Note the enamel hypoplasia, "x," at point where the primary hypocalcified stripe, PR, begins. Note the deep stain of the secondary hypercalcified stripe, SE. D = normal calcified dentin; EP = enamel epithelium; ES = enamel space; OD = odontoblasts; OE = organic enamel matrix; PU = pulp. × 2.24.

FIG. 7. Photomicrograph of area indicated in Fig. 6 and magnified. Note the partial obliteration of the primary hypocalcified stripe, PR, and compare

with the same stripe in Fig. 8. D = normal calcified dentin; OD = odontoblasts; OE = organic enamel; P = predentin; PU = pulp; SE = secondary hypercalcified stripe. × 210.

FIG. 8. Photomicrograph of area indicated in Fig. 6 and magnified. Note the prominent primary hypocalcified, PR, and secondary hypercalcified, SE, stripes in the dentin. Note the dentin, D, that was calcified before the experiment began. The width of the dentin (90 μ) laid down during the experiment approximates the amount of dentin that would be laid down normally in 6 days, the duration of the experiment. OD = odontoblasts; P = predentin. × 210.

FIG. 9. Photomicrograph of region near the anterior end of the pulp of upper incisor of Rat 301, which was given 4 injections of parathyroid hormone. Note the primary injection stripe, PR, the secondary injection stripe, SE, which is interrupted by an eosin-staining stripe and severely disturbed in its portion toward the pulp, as evidenced by the predentin, P, which is abnormal in width, irregular in its course and contains vascular inclusions. Compare with Figs. 6, 7 and 8 taken from the same histological preparation. D = normal calcified dentin; OD = odontoblasts; PU = pulp. × 210.



Schour, Tweedy and McJunkin

Effect of Parathyroid Hormone on Dentin

PLATE 90

FIG. 10. Photomicrograph of section near the lingual alveolar crest of upper incisor of control Rat 309. Note the rich number of osteoblasts, OB, lining the bone marrow space, BM. Compare with Fig. 11. × 101.5.

FIG. 11. Photomicrograph of section near the lingual alveolar crest of upper incisor of Rat 306, which received 14 injections of parathyroid hormone. Note the absence of osteoblasts; the fibrous change of the bone marrow, FM; the presence of a number of osteoclasts, OC. Compare with Figs. 10 and 13. × 101.5.

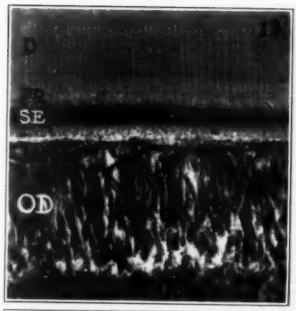
FIG. 12. Photomicrograph of a longitudinal section of midregion of upper incisor of Rat 302, which was given 4 injections of parathyroid hormone and was killed 19 hours after the last injection. Note the primary, PR, and secondary, SE, injection stripes. D = dentin which is normal and which was calcified before the experiment began; OD = odontoblasts. × 196.

FIG. 13. Photomicrograph of dentin of midsection of incisor of Rat 306, which received 14 injections of parathyroid hormone. Note the stratification, ST, which is prominent in the portion of the dentin extending over 290 μ, which approximates the amount of dentin that is laid down normally in 18 days. Compare with the preëxperimental dentin, D. OD = odontoblasts; P = predentin. × 98.

FIG. 14. Photomicrograph of the dentin of the floor of the pulp chamber of the first lower molar of Rat 323, which received 9 injections of 50 units each of parathyroid hormone. Note the eosin-staining dentin stripe, S, situated within the dentin, D, and the abnormally wide predentin border, P, next to the pulp, PU. × 210.

FIG. 15. Photomicrograph of transverse section of lower incisor of Rat 324, which was given 4 injections of 50 units of parathyroid hormone. Note the constant primary hypocalcified stripe, PR, and the disturbance and irregularity in the secondary stripe, SE, which at the top of the field is poorly calcified and contains cellular inclusions. D = dentin calcified before the experiment began; OD = odontoblasts; PU = pulp. × 210.


10
PDM
BM
OB
OB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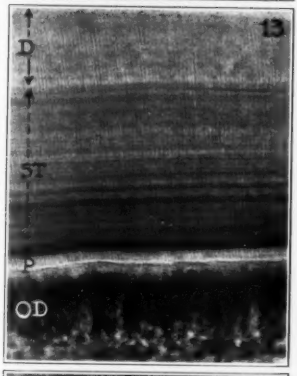
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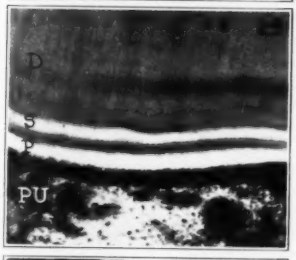
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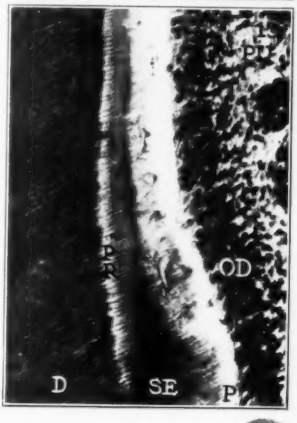
PU
OD
D
SE
P

MICROGLIA-LIKE CELLS AND THEIR REACTION FOLLOWING INJURY TO THE LIVER, SPLEEN AND KIDNEY *

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Certain cells in the nervous system which had hitherto been difficult to stain were included by Cajal[1] in 1913 under the term "third element" of the central nervous system, and were suspected by him to be of mesodermal origin. Somewhat later del Río-Hortega[2-5] was able to show that this "third element" consisted of two entirely different kinds of cells: one kind was the oligodendroglia of ectodermal origin; and the other kind he called microglia, the latter, in his opinion, being of mesodermal origin. The central nervous system was made up, therefore, of neurones (the first element), neuroglia consisting of astrocytes and oligodendroglia (the second element), and microglia (the third element). Del Río-Hortega's papers describing the microglia were published between 1919 and 1921. Today del Río-Hortega[6] believes, along with others, that the microglia represents the reticulo-endothelial system in the central nervous system.

In 1921 del Río-Hortega and Jiménez de Asúa[7] demonstrated phagocytic cells in tumors, tubercles, liver lesions, normal human kidney, and in lymph follicles stained by silver carbonate. In this paper the authors drew attention to their belief that phagocytosis in the nervous system is the function of the microglia and that the studies of del Río-Hortega on the microglia constitute a concrete example of the general problem of the histogenesis of the macrophages.

In 1927 Jiménez de Asúa,[8] using silver carbonate, demonstrated macrophages in a normal spleen and in tumors, and stated that in morphology, staining properties and in function they resemble the microglia of the nervous system and, finally, that microglia cells are members of the reticulo-endothelial system.

Cone,[9] in 1928, using del Río-Hortega's method for microglia, illustrated a phagocyte with a close resemblance to transitional microglia in a degenerating area of a hypernephroma.

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Dorothy Russell [10] in 1929 demonstrated intravital staining of microglia with trypan blue and concluded that such intravital staining identifies microglia with the rest of the reticulo-endothelial system and that microglia is a mesodermal element.

Wells and Carmichael [11] in 1930 made a study of microglia by means of tissue culture and vital staining and concluded that microglia is of mesodermal origin and is analogous to the resting histiocytes or fixed macrophages of the reticulo-endothelial system. They also found microglia-like cells in cultures from embryonic chick periosteum and limb-bud and found that these cells reacted to vital dyes *in vitro* in the same manner as wandering cells.

Visintini [12] in 1931 demonstrated microglia-like cells in the heart, voluntary muscle and urinary bladder by the method of Bolsi.

Belezky [13] in 1931, using silver carbonate, demonstrated cells in the spleen which he regarded as reticulo-endothelial cells. These cells, in our opinion, resemble microglia cells.

The microglia cells appear in the nervous system at about the time of birth. They migrate to all parts of the brain and spinal cord and remain there inactive until the advent of some disease. When this occurs the first pathological change that can be noticed in certain types of lesions is the sudden and tremendous activity of the microglia. They multiply, migrate in great numbers to the damaged area and become phagocytic, devouring the broken-down nervous tissue and other débris and apparently removing it to the nearest perivascular spaces, where they appear in the form of large, rounded, compound granular corpuscles loaded with fat (*Gitterzellen*). Thus they clear the way for the astrocytes to lay down a scar in place of the destroyed tissue.

A similar phenomenon might occur in other tissues of the body. With this possibility in mind experiments were performed on rabbits, using del Río-Hortega's original silver carbonate method of specific staining for microglia in the liver, spleen and kidney after producing a destructive lesion in these organs.

Under sterile conditions a cerebral hemisphere, the liver and the spleen of a rabbit were punctured by a hot trochar, ether anesthesia being used. At the end of 4 days the animal was killed and the brain, liver and spleen were fixed in Cajal's formol-bromide solution for about 24 hours. Frozen sections were cut from the traumatized portions of each of the three organs and stained at the same time by

del Río-Hortega's original silver carbonate method for microglia. In Figure 1 the area of brain destroyed by the hot trochar is surrounded by great numbers of microglia cells, forming a dark ring about the necrotic area. Figure 2 is a high power view of a portion of this ring. The microglia cells can be seen in different stages of metamorphosis from the almost normal cell with its spiked processes to the completely formed compound granular corpuscle loaded with fat. Figure 3 is a low power photomicrograph of a portion of the lesion in the liver. The margin of the necrotic area is marked by a dark ring of cells which in the high power view (Fig. 4) closely resemble the transitional microglia of the brain. These cells contain droplets of fat. Cells of the same character are present at the margin of the puncture wound of the spleen. The stages in the transformation of a cell with spiked processes into a swollen, rounded form are illustrated in Figures 5 to 10 by camera lucida drawings of six cells found at the margin of the necrotic area in the same spleen. All of the cells except the first contain droplets of fat, which appear black in the picture. We wish to draw attention to the close resemblance of the cells in this figure to the microglia cells illustrated in Plate 91 of Dorothy Russell's article, referred to above.

Using the same technique as in the first experiment a kidney of a rabbit was punctured by a hot trochar. The animal was killed at the end of 4 days and the injured organ was fixed in formol-bromide. Sections of the lesion were cut and stained in the same manner as were the brain, liver and spleen. Figure 14 is a high power view of fat-containing microglia-like cells in the process of swelling at the margin of the damaged area in the kidney. Some of these cells are elongated and have small spikes, very much like the microglia cells in a paretic brain (the "rod-cells" or *Stäbchenzellen* of general paralysis).

A few hours before a puncture wound of the spleen was made 10 cc. of a 1 per cent aqueous solution of trypan blue were injected into an ear vein of a rabbit. The animal was allowed to live 4 days, and during this period 35 cc. of the same solution of trypan blue were injected intraperitoneally. The spleen was fixed and sections of the lesion were cut and stained as before, with the exception that the silver impregnation was not toned in gold chloride because it tended to change the blue dye to purple. Figure 11 is a low power view of the damaged area outlined by a well marked dark ring of cells.

In the high power view of a portion of the ring (Fig. 12) many swollen cells, which closely resemble pathological microglia, can be seen, and all of the cells in this picture contain trypan blue in granular form. Figure 13 is a high power photomicrograph of a group of cells in a splenic nodule at some distance from the lesion. These cells closely resemble slightly swollen microglia, and small spike-like projections on their main processes can be seen, a feature characteristic of the microglia of the nervous system. Trypan blue could not be demonstrated in this group of cells. By staining two consecutive sections of the block, one with silver carbonate and the other with hematoxylin and eosin, the opposite halves of the same cells impregnated with silver were colored by the organic dyes. Stained by the more familiar method the cells pictured in Figure 12 have the following characteristics. Compared with the nucleus of the lymphocyte the nucleus of the argyrophilic cell is larger, less deeply stained by hematoxylin, and varies in shape, tending to be rounded, oval or kidney-shaped. It contains a nucleolus and a uniform distribution of fine granules of chromatin. The clear cytoplasm is pale pink, almost colorless, and contains large and small granules of trypan blue, masses of amber blood pigment and an occasional engulfed lymphocyte. The shape of the cell is as varied as the shape of the nucleus and its processes, which are so clearly impregnated with silver, cannot be distinguished in the section stained with hematoxylin and eosin.

The next step was to determine whether microglia-like cells are present in the normal liver, spleen and kidney as they are found in a resting state in the normal nervous system and whether they could be demonstrated by the same technique of staining. Accordingly, the liver, spleen and kidneys of normal rabbits were stained by del Río-Hortega's original silver carbonate method for microglia. Figure 15 is a high power photomicrograph of the normal liver of a rabbit. In the center of the field there is a triangular-shaped cell with three long processes extending between the liver cells. Typical of many others scattered throughout the liver and undoubtedly representing the nearly normal or very early transitional form of the cells demonstrated in Figures 3 and 4, it is morphologically similar to the microglia cells of the nervous system. In Figure 16, a high power photomicrograph of the normal spleen of a rabbit, one slightly swollen microglia-like cell is seen at the edge of a splenic nodule.

This cell is typical of many others found at the periphery of the nodules and these undoubtedly are the source of such cells as those demonstrated in Figures 5 to 10, 11 and 12. Figures 17 and 18 are high power photomicrographs of microglia-like cells in a normal kidney of a rabbit. Spike-like projections on their processes are well shown. Many similar cells were found scattered between the tubules throughout the kidney and they are undoubtedly the resting or early transitional forms of the cells demonstrated in Figure 14. In the normal organs that were studied all of the microglia-like cells were somewhat swollen, as if they were constantly being stimulated to activity.

Summary

1. Cells have been demonstrated by del Río-Hortega's original silver carbonate method of specific staining for microglia in the liver, spleen and kidney of the rabbit that in morphology are identical with the nearly normal or very early transitional forms of microglia in the nervous system.

2. In their reaction to injury and to the intravital injection of trypan blue they have been shown to be identical with microglia.

3. These cells have been demonstrated in a transitional stage with spiked processes like microglia and containing droplets of fat or granules of trypan blue.

4. By the silver carbonate method of staining earlier transitional forms have been demonstrated that contain no visible amounts of fat or trypan blue.

5. A more advanced transitional form has been shown in preparations of the spleen of the rabbit to be a histiocyte or large mononuclear phagocyte without processes and containing droplets of fat, granules of trypan blue, blood pigment and engulfed lymphocytes.

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DESCRIPTION OF PLATES

PLATE 91

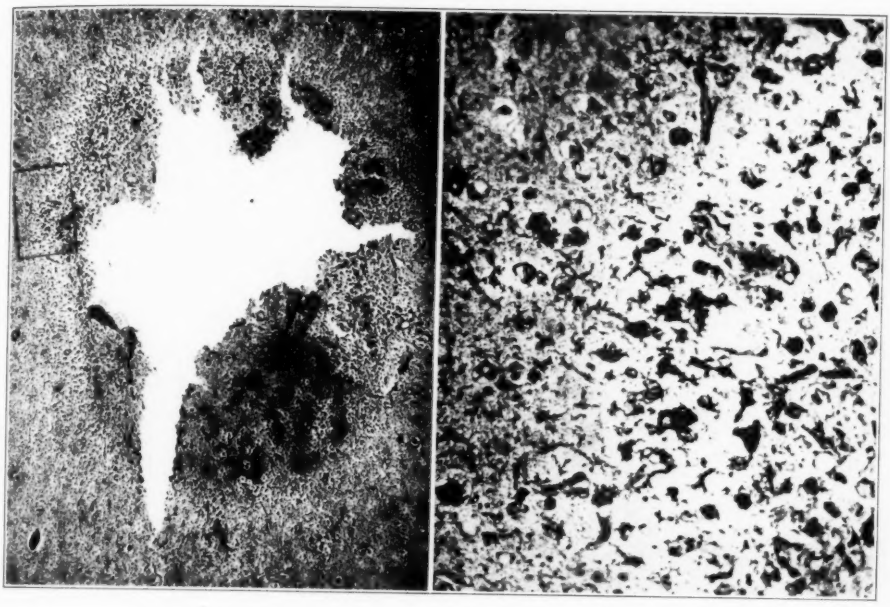
We are indebted to Mr. William S. Dunn for the photomicrographs illustrating this paper.

FIG. 1. Microglia cells in the cerebrum of a rabbit forming a dark ring about a necrotic lesion produced by puncture with a hot trochar. Silver carbonate stain for microglia. × 21.

FIG. 2. A high power view of cells in the area of Fig. 1 outlined in black, showing the transformation of cells with spiked processes into rounded forms filled with droplets of fat. Silver carbonate stain for microglia. × 176.4.

FIG. 3. A portion of a necrotic lesion in the liver of a rabbit produced by puncture with a hot trochar. Microglia-like cells are gathered at its margin. Silver carbonate stain for microglia. × 46.20.

FIG. 4. A high power view of cells in the area of Fig. 3 outlined in black, showing cells with spiked processes and larger, irregular forms containing droplets of fat. These cells resemble the microglia at the margin of the lesion in the brain. Silver carbonate stain for microglia. × 149.24.



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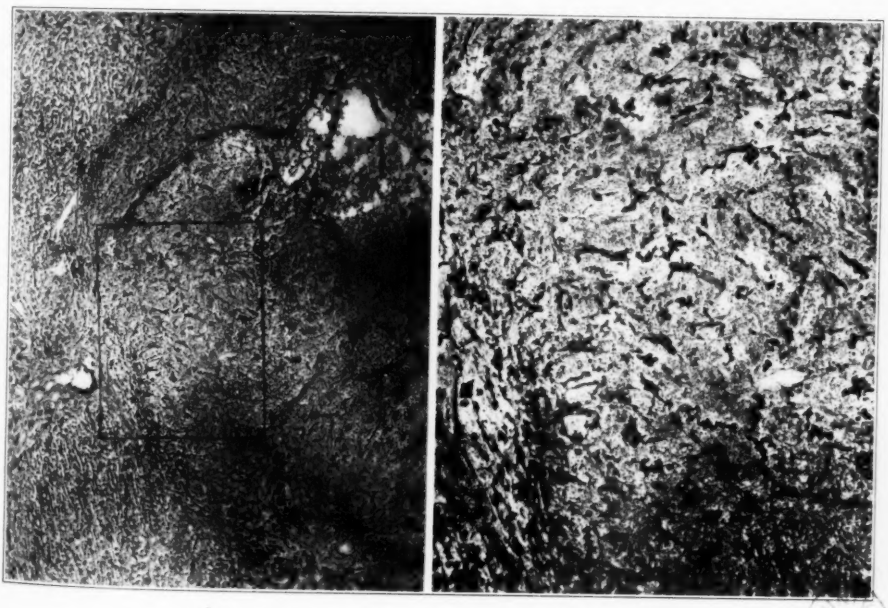

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Dunning and Stevenson Microglia-like Cells

PLATE 92

FIGS. 5–10. Camera lucida drawings of six cells found at the margin of a necrotic area produced by a hot trochar in the spleen of a rabbit, illustrating the stages in the transformation of a microglia-like cell with spiked processes into a large rounded form. Note the apparent transition of the processes into rounded projections resembling pseudopodia. All of the cells except the first contain droplets of fat, which appear black in the picture. Silver carbonate stain for microglia with Sudan III. × 950.

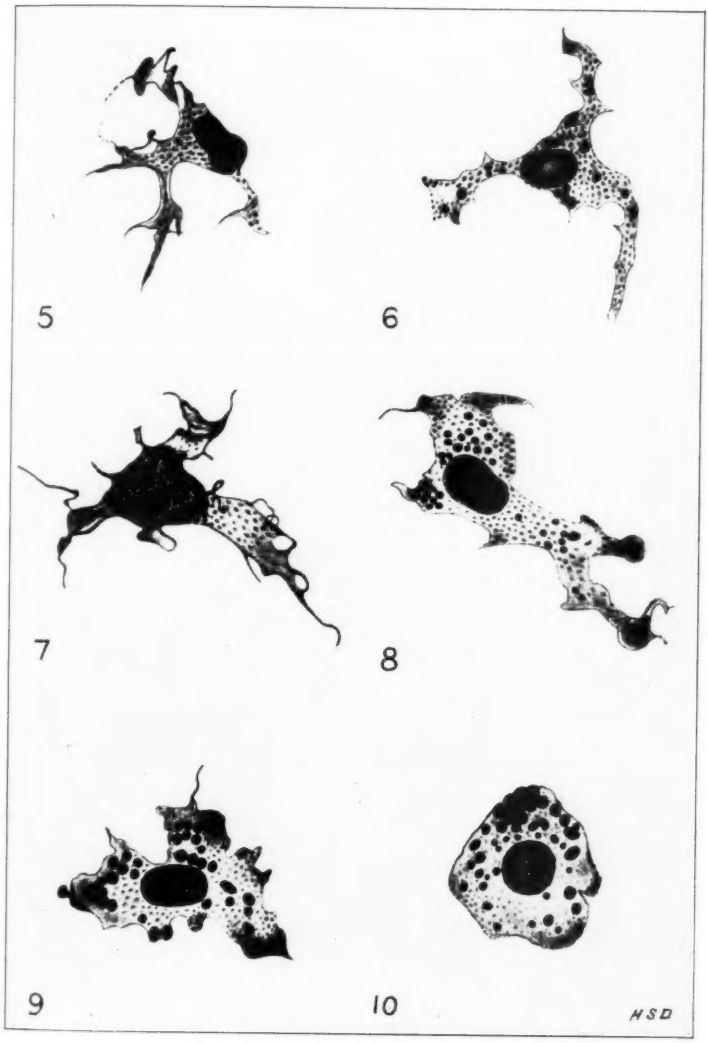
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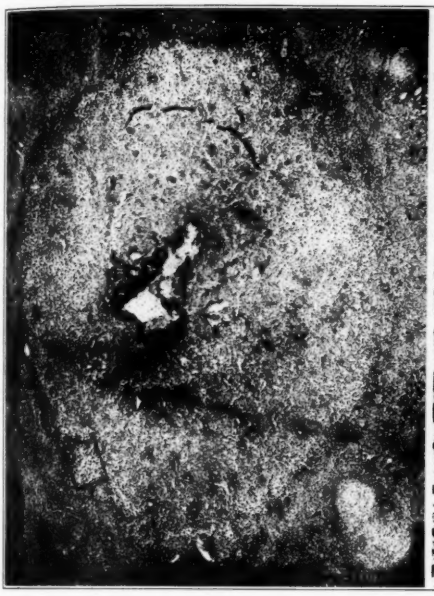
PLATE 93

FIG. 11. A necrotic lesion outlined by a dark ring of cells produced by a hot trochar in the spleen of a rabbit injected intravitally with trypan blue. Intravital trypan blue and silver carbonate stain for microglia. × 12.6.

FIG. 12. A high power view of cells at the margin of the lesion in Fig. 11 in the area outlined in black. Granules of trypan blue are present in microglia-like cells with spiked processes and in the larger rounded forms. Intravital trypan blue and silver carbonate stain for microglia. × 149.24.

FIG. 13. Cells resembling slightly swollen microglia in a splenic nodule at a distance from the lesion in the spleen pictured in Fig. 11. Note the numerous spikes on their main processes. Trypan blue could not be demonstrated in this group of cells. Intravital trypan blue and silver carbonate stain for microglia. × 149.24.

FIG. 14. A high power view of fat-containing microglia-like cells in the kidney of a rabbit gathered at the margin of a necrotic lesion produced by a hot trochar. Note the elongated forms with spikes resembling the rod-cells in a paretic brain. Silver carbonate stain for microglia. × 126.



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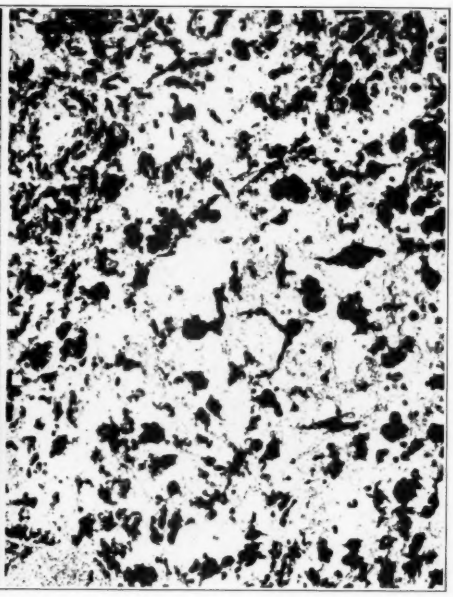

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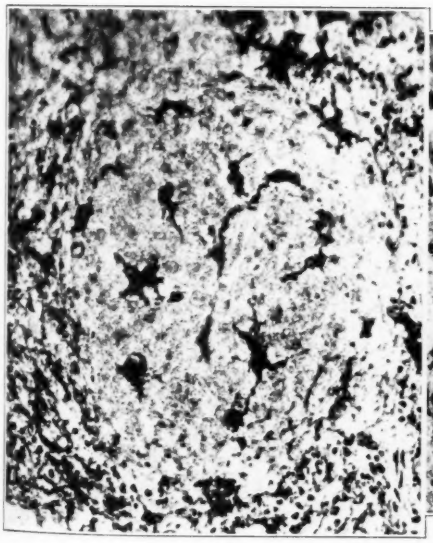

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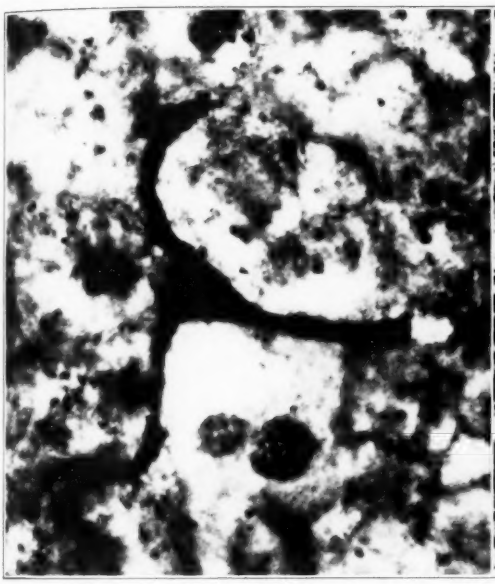
PLATE 94

FIG. 15. A cell in the liver of a normal rabbit resembling a nearly normal or very early transitional microglia cell. Note the manner in which its three processes insert themselves between the liver cells. Silver carbonate stain for microglia. × 950.

FIG. 16. A cell resembling a slightly swollen microglia cell at the edge of a splenic nodule in the spleen of a normal rabbit. Silver carbonate stain for microglia. × 600.

FIG. 17. A cell resembling an early transitional microglia cell in the kidney of a normal rabbit. Silver carbonate stain for microglia. × 1200.

FIG. 18. Another cell in the kidney pictured in Fig. 17 resembling a nearly normal or very early transitional microglia cell. Note the spikes on its main process and its position between the kidney tubules. Silver carbonate stain for microglia. × 950.



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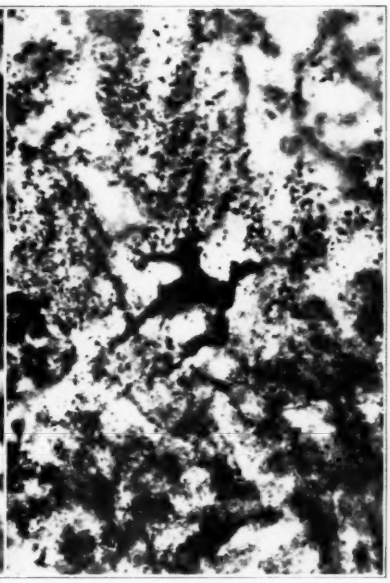

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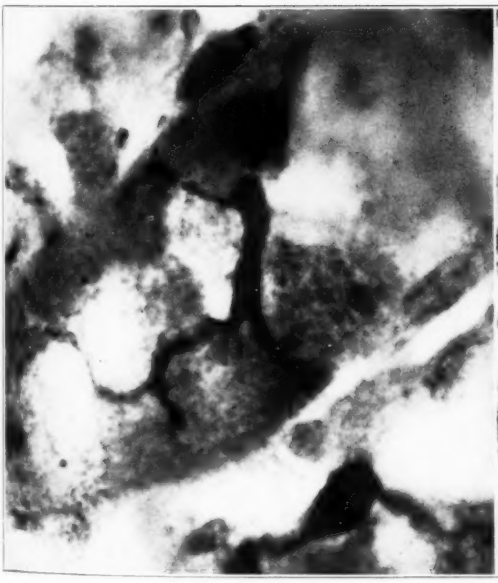

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POLYARTERITIS NODOSA *

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NOMENCLATURE

In 1865 Kussmaul and Maier [1] described an arteritis characterized by the formation of multiple circumscribed nodulations in the small arteries of various parts of the body. They named it periarteritis nodosa, a term that implies inflammatory changes in and around the adventitia of arteries with the production of nodules, and one that was intended as a précis of the morphological peculiarities of the condition. We now know that the inflammation is by no means confined to the adventitia, that the primary changes are probably in the media, and that the disease usually affects a large number of vessels. In recognition of these facts Dickson [2] suggested the name polyarteritis nodosa, which is a more accurate epitome of what we know of the condition, and which seems to us preferable to the older term.

INCIDENCE

Approximately 150 cases of polyarteritis nodosa have been reported, less than 20 of them in the American literature (although in this country a number of excellent summaries have appeared, notably those of Ophüls,[3] and Lamb [4]). The greater number of routine autopsies in European clinics partly explains the relative infrequency with which the disease is recognized in this country, for ante mortem diagnosis is made in not more than 20 per cent of cases. However, the lesion is rare even when histological studies are made in a large series of autopsies. In 2035 autopsies at the Peter Bent Brigham Hospital Bennett and Levine [5] found only 2 cases. At the Los Angeles County Hospital, during the past 15 years (1918–1933), there have been 10,000 autopsies, only 1 of which showed the classical pathological findings of polyarteritis nodosa.

* Received for publication October 17, 1933.

It is conceivable, of course, that there may be a mild or early form of the disease in which the arterial changes are so slight as to escape detection in the routine microscopic study of autopsy sections. Clinically there can be no doubt that many cases have been completely unsuspected or misinterpreted, for polyarteritis nodosa habitually masquerades as one of the commoner infectious diseases. It is generally believed to be almost uniformly fatal, usually in the course of a few weeks, but the high mortality and the low incidence may both be explained partly by the fact that with few exceptions only the fatal cases have been recognized. Some authors believe that the total number of recorded instances grossly underestimates the actual frequency of the malady, and that many mild cases have undergone spontaneous recovery.

Etiology

Of the many propounded theories of etiology none has been generally accepted. For some time there was a tendency to regard the disease as a sort of aberrant manifestation of syphilis or some other systemic infection. The present tendency is to regard polyarteritis nodosa as a disease in its own right, quite independent of any underlying condition, and incrimination of syphilis seems utterly without foundation.

The febrile course and other outstanding symptoms are those commonly associated with sepsis and there is every reason to suspect an infectious origin. The small blood vessels in other infections, such as the cerebral vessels in influenza and the skin capillaries in epidemic meningitis, may show comparable lesions, and Bennett and Levine [5] refer to the similarity between the pathological findings in polyarteritis nodosa and those of Rocky Mountain spotted fever and typhus fever, as discussed by Wolbach,[6] and Wolbach, Todd and Palfrey.[7]

Assuming that we are dealing with an infectious disease, we still have to decide whether there is a specific infective agent, or whether a variety of agents may produce the same striking vascular phenomena. Spiro [8] does not consider polyarteritis nodosa a disease *sui generis*; he thinks it merely one of the forms that may be taken by a mesarteritis due to a variety of infections. Gruber [9, 10] states: "We regard periarteritis nodosa as the expression of a con-

stant characteristic reactive process of the arterial system in the manner of an hyperergic phenomenon during the course of very different infectious-toxic diseases. This is hypothesis!" However, when we contrast the enormous number of infections with the rarity of the histological phenomena seen in polyarteritis nodosa, it is difficult to believe that the vessels are capable of such a constant specific response to non-specific excitants.

Evidence is accumulating in favor of the specific infectious nature of the disease. Harris and Friedrichs,[11, 12] though their experiments have not been widely accepted, believe they have produced the disease in animals. Other workers have observed in the lower animals (deer, calf, pig, dog) lesions almost identical with those we see in man. Altogether, the probabilities are that this peculiar and unmistakable lesion is induced by a specific infectious agent, probably a filterable virus with a predilection for the arterial system.

Classification

Obviously there can be no etiological classification. Histologically the lesions are readily grouped according to the stage of advancement of the inflammatory process. Clinically the victim's progress will depend upon the anatomical locations of the lesion and the severity of the arterial damage in these locations, so that we may attempt to divide polyarteritis nodosa into different types, according to predominant symptoms or the rapidity of its course. Sometimes the most battle-scarred areas may be mirrored in the clinical picture, but often the autopsy findings are the only reliable evidence upon which we may say that the disease is renal, cardiac, cerebral, abdominal, dermatological or neuromuscular in type.

Pathology

The affected vessels may often be recognized in the gross by the multiple small (peas-in-a-pod) nodules scattered along their course. These nodules may be inflammatory foci or actual aneurysms. Microscopically the periarterial connective tissue, the adventitia and the media show dense cellular infiltration consisting largely of polymorphonuclear leukocytes, and the media often shows extensive necrosis which may give rise to aneurysms. The intima shares the inflammatory process and, if the endothelium and internal elastic

lamina are destroyed, thrombosis results. Vascular occlusion with consequent infarction of the organs supplied by the affected vessels is the pathological key to the sudden critical clinical symptoms that may appear in the course of the disease. These vary according to the location and size of the occluded vessels.

The selection of the media rather than the adventitia as the primary site of attack (contrary to earlier conceptions) was demonstrated by Fishberg [13] in a patient who suffered from an acute form of the disease and died in a few days. The vessels affected are the small or medium sized muscular type arteries, while the elastic type escape. Arkin [14] says that the organs most frequently involved are the kidneys (80 per cent), heart (70 per cent), liver (65 per cent), gastro-intestinal tract (50 per cent), pancreas (25 per cent), mesenteric artery (30 per cent), muscles (30 per cent), and peripheral nerves (20 per cent). The central nervous system is attacked in 8 per cent of the cases. The disease may confine itself to one organ for considerable periods of time, and involvement of other organs is irregular and wholly unpredictable. Sometimes the characteristic nodules appear in the skin, and a diagnosis is then readily made by excision of one of these lesions.

In his illuminating analysis of the pathological findings Arkin divides the disease into four stages: (1) the alterative-degenerative or beginning stage; (2) the acute exudative inflammatory stage; (3) the granulation tissue stage; and (4) the healed end-stage, or scar tissue stage. Of course, these stages are no more sharply separable than those of any other inflammatory process. They may merge and vary enormously in each individual case, and sometimes may telescope into one short fulminating bout in which all the stages seem to occur almost simultaneously with terrifying despatch.

Clinical Syndrome

The victim of polyarteritis nodosa is usually a man (males predominate four to one) between 10 and 50 years of age (the youngest reported case was 3 months, the oldest 78 years of age). The typical onset is said to be acute, with a short septic course ending in death (often due to hemorrhage from a ruptured aneurysm) in a few weeks. However, more often than most textbooks quote, the onset is gradual and insidious, the course subtle, capricious and relatively pro-

longed. Arkin's case of histologically healed polyarteritis nodosa lived 4 years after his single attack of acute illness.

Evidences of sepsis are the rule — fever, high leukocytosis (and in our case eosinophilia), anemia, prostration and sometimes splenic tumor. Concomitant with these there are other more variable signs and symptoms that reflect the hidden vascular insults. The cardiac type behaves like a case of coronary sclerosis and there is apt to be an anginoid syndrome with manifestations of myocardial insufficiency. When the kidney is chiefly involved the course is often indistinguishable from essential hypertension with nephrosclerosis. There may be hypertension, visual disturbances and renal insufficiency, and if infarction occurs there is likely to be sudden hematuria. When the mesenteric vessels are occluded the symptoms may simulate those of an acute abdominal condition, and differential diagnosis is extremely difficult when the surgeon is confronted with a patient who has abdominal pain, fever, leukocytosis, nausea and vomiting. Neuromuscular symptoms of pain and tenderness along the peripheral nerves and in the muscles occur to some degree in most forms of the disease. It has been supposed that the neuritis was always secondary to changes in the arteries accompanying the nerves, but Carr's case [15] seems to confirm the work of other investigators (*e.g.*, Wohlwill,[16]) who believe that there may be severe parenchymal nerve degeneration with or without arterial damage. Fletcher,[17] Dickson,[2] and Bennett and Levine [5] reported cases of the cerebral type of polyarteritis nodosa, and Bennett and Levine's second patient developed a meningitis, during the active stage of which there was an increased number of polymorphonuclear leukocytes in the cerebrospinal fluid, though no organisms could be detected.

Differential Diagnoses

Small wonder that with such bizarre manifestations polyarteritis nodosa with monotonous regularity should pass wholly unsuspected (in about 88 per cent of cases probably) before autopsy. In our case, as in many others, even autopsy failed to reveal the condition until tissue sections were seen under the microscope.

During life the disease is usually mistaken for neuritis, myositis, trichinosis, vascular nephritis, typhoid fever, miliary tuberculosis, gastro-enteritis, pyemia, purpura hemorrhagica, hemorrhagic ne-

phritis, endocarditis, or acute abdominal conditions. It would be academic to point out features that might distinguish polyarteritis nodosa from each of these conditions. Indeed, it is seldom necessary or possible to make a careful differentiation. The diagnosis is usually unprovable but the possibility of a common vascular cause should be considered whenever a patient with sepsis exhibits a wide variety of symptoms, not peculiar to a specific disease. When an obscure sepsis cannot be fitted into one of the commoner infectious groups, the internist should consider polyarteritis nodosa as a possible diagnosis.

The following case is reported because it presents some peculiar features of a rare condition.

Report of Case

Clinical History: A male negro, aged 49 years, was admitted to the Los Angeles County Hospital Jan. 15, 1932, with a diagnosis of influenza. He complained of headache, pain in the shoulders and neck, chills, fever and cough. His temperature was 104, pulse rate 100.

In childhood the patient had had measles, mumps, chickenpox and pertussis. He remembered no other illness until Nov. 21, 1931, when he was ill with what he thought was the "flu." He recovered and went to work for a while but in January had similar complaints. He denied venereal disease. By occupation he was a carpenter. He had never used alcohol or tobacco. His father, mother, brother and three sisters were living and well.

Examination revealed a well nourished and well developed negro with a blood pressure of 135/90. There were no significant findings except several carious teeth, tenderness and pain on pressure along the course of the left spinal accessory nerve, and a temperature that fluctuated between 99 and 102 F. The pulse was from 88 to 100.

Blood and spinal fluid Wassermanns were negative. The white blood count was 11,300, polymorphonuclear leukocytes 82 per cent. Urine negative.

The patient left the hospital Feb. 11, 1932, with a diagnosis of left spinal accessory neuritis, radiculitis and pharyngitis.

On May 18, 1932, the patient returned to the hospital complaining of pain in the shoulders, neck, and arms. He said that he had been feeling somewhat better until Feb. 16, 1932, when in trying to stop a gun fight, he fell unconscious and remained so for 16 hours. When he recovered consciousness he found his right arm and right leg were paralyzed. In 3 days he was able to walk with a cane, but the full use of his right extremities did not return.

Physical examination at this time revealed paresis of the right leg and arm with occasional fine tremors. There was marked tenderness on attempting to palpate the right kidney. The legs and ankles were edematous. The blood pressure was 118/80, temperature 99 to 104, pulse 88 to 100.

On June 2nd a consultant noticed extreme tenderness in the right costovertebral angle and thought it significant of a pyelitis. On July 25th there was ten-

derness on palpation in both lumbar regions. The next day the patient complained of sudden, severe abdominal pain for which no cause could be detected. On Oct. 3rd he was suddenly stricken with agonizing precordial pain which radiated down the left arm. Morphine was administered and the pain was relieved in about half an hour. The next day there was still residual precordial pain, but not nearly so severe as on the previous day.

Laboratory Findings: Wassermann negative; urine 30 pus cells per field, few casts; basal metabolic rate +6; blood smears negative.

The blood counts were as follows:

May 18, 1932, red blood cells 4,250,000, hemoglobin 75 per cent, white blood cells 7000, polymorphonuclears 60 per cent, eosinophiles 27 per cent, mononuclears 5 per cent, basophiles 3 per cent.

July 26, 1932, red blood cells 3,220,000, hemoglobin 47 per cent, white blood cells 12,500, polymorphonuclears 64 per cent, eosinophiles 15 per cent, lymphocytes 16 per cent, mononuclears 5 per cent.

Aug. 23, 1932, white blood cells 16,700, polymorphonuclears 38 per cent, eosinophiles 33 per cent, lymphocytes 27 per cent, mononuclears 2 per cent.

X-ray examination on Aug. 22, 1932 showed no parenchymal pathology in either lung. Moderate enlargement of the left ventricle consistent with hypertensive heart disease was present.

On May 24, 1932, kidney, ureters and bladder studies were not significant of any renal lesion.

On Oct. 8, 1932, the patient left the hospital against his physician's advice. At least twelve special consultants had examined him but no satisfactory diagnosis could be agreed upon. Suggestions included coronary sclerosis, subdiaphragmatic abscess, echinococcic liver cyst, tuberculosis, coccidioidal granuloma, pyelonephritis, Malta fever. Practically all of these possibilities were ruled out conclusively while the patient was hospitalized.

On Nov. 23, 1932, the patient was readmitted for the third time and was brought to the hospital in a comatose condition. He was emaciated, extremely dyspneic and his heart tones were barely audible. He died 1 hour after admission.

Summary of Autopsy

About the base of the cerebellum and around the brain stem the leptomeninges were greatly thickened. The affected area had a greenish gray color that gave it the appearance of being an acute process superimposed upon a more chronic lesion. The entire area was limited to the base of the brain and was thus quite similar to a tuberculous meningitis, but careful search revealed no miliary tubercles.

A few pleural adhesions were found at the left apex but no tuberculosis was in evidence. There was marked edema of the lungs.

A recent fibrinous pericarditis involved the entire pericardial sac and considerable serosanguinous fluid was present. The heart weighed 460 gm., the increase in size being due to left ventricular

hypertrophy. The myocardium was light brown in color and no areas of fibrosis were found. The aortic cusp of the mitral valve had a soft vegetation 5 mm. in diameter attached to the line of contact. The coronary arteries were quite markedly sclerotic but not occluded.

A small infarct was found in the spleen.

The kidneys presented a striking appearance. Each weighed 290 gm., and on section showed a diffusely granular appearance. The capsule was adherent and when stripped left a granular surface. The cortex was thicker than usual.

The adrenals, pancreas, gastro-intestinal tract, bladder and prostate presented no evidence of gross pathology.

A smear taken from the meninges showed numerous pneumococci.

Microscopic Examination

Meninges: There is a marked acute purulent meningitis which overshadows any other pathological condition that might be present.

Heart: The pericardial surface is greatly thickened and infiltrated with large numbers of polymorphonuclear leukocytes, plasma cells and eosinophiles. A small amount of fibrin is present. The small branches of the coronary arteries show a striking change. There is considerable periarterial cellular infiltration of leukocytes, including eosinophiles and plasma cells, which in many instances takes a peculiar bipolar arrangement seen in the photomicrographs. Even more striking is the marked medial and intimal thickening with almost total occlusion of the vessel lumen. The myocardial fibers show surprisingly little evidence of degeneration.

Kidneys: The process in the kidneys is similar to that in the heart but much more pronounced, so that the entire interstitial structure is infiltrated with numerous polymorphonuclear leukocytes, eosinophiles, and fewer plasma cells. The glomeruli and tubules show little change. The arterioles of all sizes are involved and there is more periarterial infiltration than was seen in the heart. Another feature not seen in previous sections is the presence of numerous giant cells around the vessels. These are small in size and contain six to eight relatively large nuclei. The destruction and separation of the various layers of the media are especially well demonstrated in this section.

Liver: Only a few of the larger arterioles show characteristic changes.

Spleen: A typical anemic infarct is found. There is no evidence of polyarteritis.

Pancreas: In the section studied there is one small artery showing changes similar to those noted in the heart and kidney.

Pathological Diagnosis: From the microscopic examination, which, unfortunately, is somewhat incomplete (due to the diagnosis not being suspected at autopsy so that only routine tissue blocks were saved for microscopic examination), this is a case of polyarteritis nodosa affecting chiefly the kidneys and the heart.

Discussion

In this case our failure to make a correct diagnosis of the condition present is instructive enough to deserve some emphasis. Here was a man dramatically ill over a period of nearly 1 year, during most of this period surrounded with competent medical talent, and with all desirable facilities. Yet, as far as we know, polyarteritis nodosa was not once mentioned as a possible explanation of his illness. Even if it had been considered it is doubtful if the diagnosis would have been verified ante mortem, or if the course of the disease could have been altered by its recognition. But this does not justify our failure to consider the possibility of polyarteritis nodosa. It is true that the disease is rare and the clinical symptoms vary, so that we cannot expect positive ante mortem diagnoses in a large percentage of cases. However, as we learn more of its behavior we should come to include it more frequently in our differential consideration of obscure sepses.

Even at autopsy the condition was not suspected. This was due to the fact that in this case only the very small arteries were involved, so that the characteristic nodulations were not noticeable in the gross. Only on microscopic examination of tissue blocks was the positive diagnosis revealed.

Arkin lists the important symptoms observed in his 5 cases as accelerated regular pulse in 5 instances, edema of the legs in 5, septic type of temperature in 4, pain in the extremities, polyneuritis in 4, hematuria in 4, cardiac insufficiency in 3, melena in 3, cerebral symptoms in 2, onset with acute angina in 2, abdominal pain in 2,

and changes in the fundus oculi in 1 case. The clinical findings in our case correspond quite closely with this list, *viz.*, accelerated regular pulse, edema of the legs, septic temperature, pain in the extremities, an acute anginoid syndrome, abdominal pain, a hemiplegic attack, costovertebral pain and tenderness, secondary type of anemia, leukocytosis with eosinophilia, and progressive emaciation.

In all probability the hemiplegic attack that occurred in February, 1932, was due to cerebral arteriopathy, which our histological investigations were not thorough enough to discover. Meningitis has been observed in polyarteritis nodosa, but in this case the meningitis was probably a terminal acute pneumococcic invasion entirely unrelated to the arteritis.

The most striking laboratory finding was the eosinophilia noted in repeated blood examinations. Eosinophilia, however, has not been stressed by other writers and probably is by no means a criterion of the disease.

Polyarteritis nodosa is generally regarded as a progressive and incurable disease. This view is supported by the rapidly fatal termination of most of the reported cases, nearly all of which reveal histological evidences of acute inflammation as well as chronic reparative changes. In spite of this dubious prognosis, there can be no doubt that occasionally the process comes to a halt. Arkin[14] described 1 case of complete histological healing. This patient suffered only one acute illness and then lived 4 symptom-free years before death.

At present it is impossible to judge if any form of therapy can retard the inflammatory changes or assist the healing processes. However, Carling and Hicks[18] used arsenical preparations intravenously and observed consequent remission of symptoms, and this was strikingly confirmed in a recent report by Schottstaedt.[19]

Summary and Conclusions

The term "periarteritis nodosa" does not accurately connote the morphological realities of the disease as we now know them. Dickson suggested "polyarteritis nodosa" as a name for this condition, which seems a more descriptive term, free of misleading implications.

A specific filterable virus with a selective affinity for the small

and medium sized muscular type arteries of the body is probably the cause of polyarteritis nodosa. Any organ or combination of organs may be affected at any time in the course of the disease, and the resulting clinical manifestations may be bizarre in the extreme. The visceral arteries are involved more frequently than those of the extremities, and the organs most commonly affected are the kidneys, heart, gastro-intestinal tract, pancreas, muscles, peripheral nerves, liver, spleen, and cerebrum.

Pathologically the inflammatory changes are not confined to the adventitia and periarterial connective tissue, as originally supposed. All the vascular coats are eventually involved and the primary changes take place in the media. Destruction of the media may give rise to aneurysm formation. Involvement of the intima with rupture of the elastic membrane may produce thrombosis. The process as a rule is progressive and in practically all of the reported cases there has been evidence of acute inflammatory changes superimposed upon the chronic reparative efforts. However, Arkin has described 1 case of histological healing and he believes that in rare instances the process may come to a complete standstill.

Polyarteritis nodosa is seldom diagnosed or even suspected before autopsy, and even at autopsy there may be no gross indications of its presence. The internist should be familiar with the cardinal symptoms of the disease and its notoriously capricious behavior. Then, when the commoner possibilities have been carefully ruled out in a patient with septic manifestations and varied symptomatology, polyarteritis nodosa should be given consideration.

Carling and Hicks, and recently Schottstaedt have reported cases in which remission of symptoms seemed to follow the intravenous administration of arsenicals.

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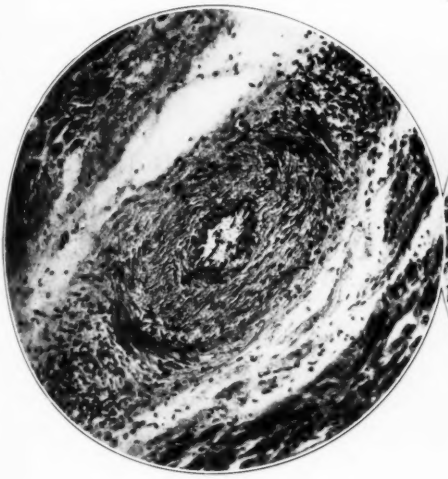
DESCRIPTION OF PLATES

PLATE 95

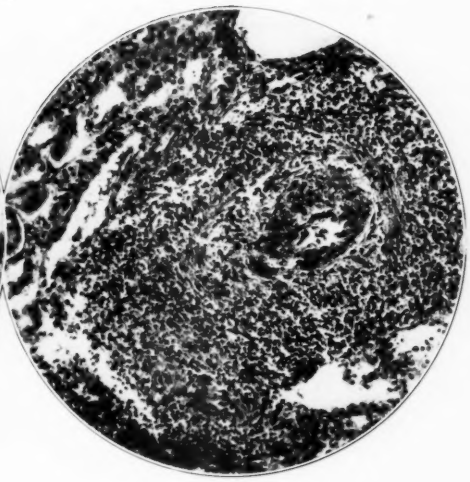
FIG. 1. Section from heart showing peculiar bipolar distribution of periarterial exudate. × 130.

FIG. 2. Arteriole of kidney showing intense cellular infiltration with separation of muscle layers. × 130.

FIG. 3. Arteriole of kidney showing marked thickening of the wall and giant cell formation. × 130.



1



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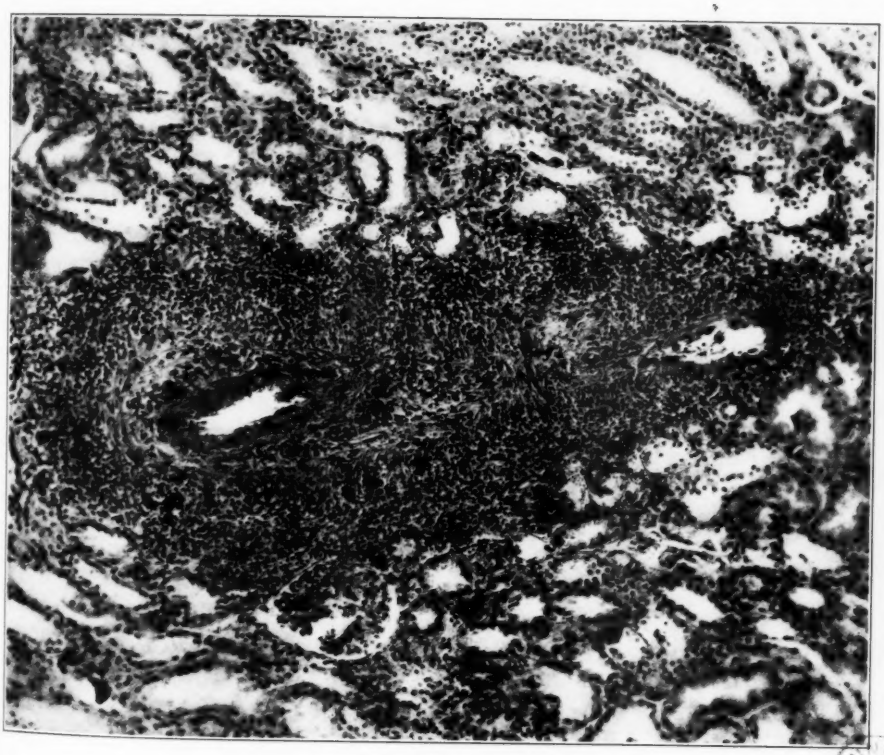

3

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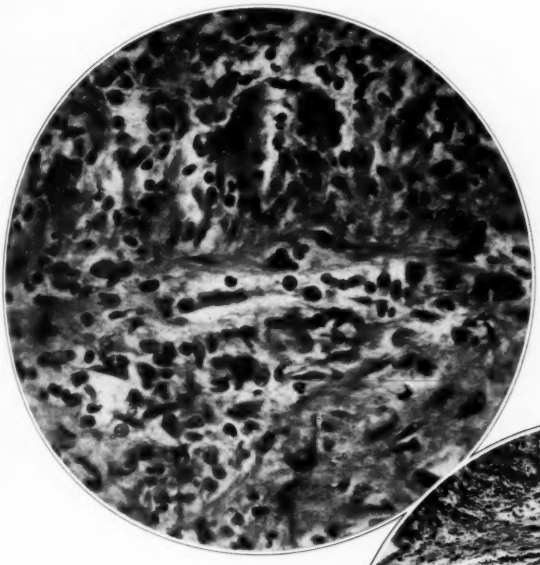
PLATE 96

FIG. 4. High power view of giant cells seen in Fig. 3. × 250.

FIG. 5. Small artery in pancreas showing extreme intimal thickening. × 130.

FIG. 6. Arteriole of liver showing extreme intimal thickening. × 130.



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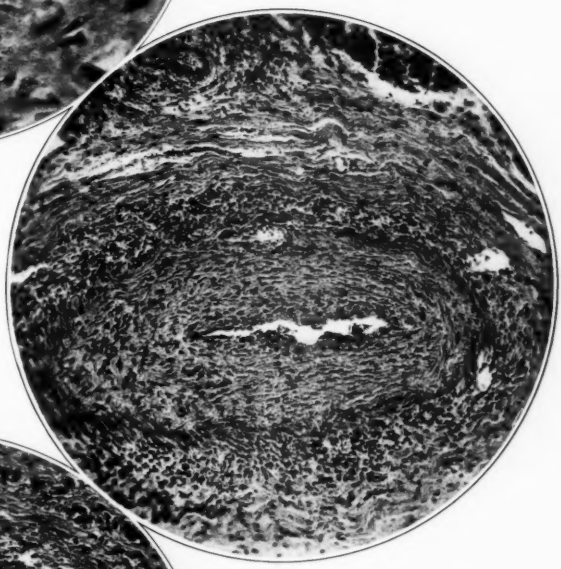
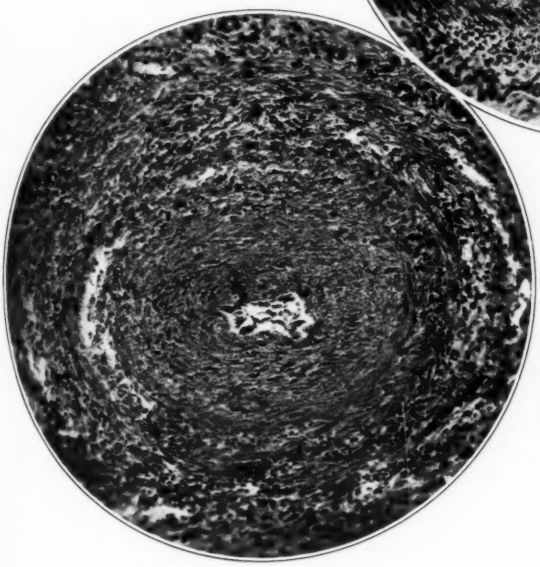


5



6

Haining and Kimball

Polyarteritis Nodosa

HISTOLOGICAL CHANGES IN THE CENTRAL NERVOUS SYSTEM FOLLOWING EQUINE ENCEPHALOMYELITIS *

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Review of Literature

Meyer, Haring and Howitt [1] have described an infectious disease in horses and mules which they demonstrated to be produced by a filterable virus, and which affects the brain and spinal cord of these animals. They reported no important gross anatomical lesions, but described significant microscopic changes in the brain and cord consisting of perivascular hemorrhages, infiltration of the sheaths of the blood vessels and scattered patches of leukocytic infiltration in the gray, and occasionally in the white, matter.

Haring, Howarth and Meyer [2] have described briefly the symptoms of this disease in horses and mules, which can be attributed directly to the microscopic changes in the brain. They stated that the malady is apparently identical with the horse disease that at different times during the past 60 years has caused heavy losses in various parts of the United States, particularly in the west central states, and which has been called Kansas-Nebraska horse plague, and also, incorrectly, cerebrospinal meningitis, forage-poisoning and botulism.

In a subsequent paper Meyer, Haring and Howitt [3] concluded that the disease, as observed in California, differed etiologically from the equine encephalitis described by Moussu and Marchand [4] in France, and from the well known Borna disease of Germany. According to Records,[5] and Records and Vawter [6] the outbreak, which started in horses and mules in California in 1930, had spread by 1932 throughout most of the western states. Newsome [7] described its occurrence in Colorado. In 1932 and again in 1933 Meyer [8, 9] summarized the existing knowledge of the disease.

Howitt [10] has shown that the virus may be recovered during the febrile period from various parts of the central nervous system and

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from the blood of experimentally infected animals, including the horse.

Records and Vawter [11] have attributed favorable results to the use of antiserums in both laboratory and field cases.

Vawter and Records,[12] Kelser,[13] and Giltner and Shahan [14] have described various ways of artificially transmitting the disease.

Recently Syverton, Cox and Olitsky [15] have shown similarities in the virus of equine encephalomyelitis and vesicular stomatitis, when inoculated into monkeys, guinea pigs and mice. They found that when such animals succumbed to experimental encephalomyelitis or vesicular stomatitis, the gross and microscopic changes in the brain were apparently identical and the same type of intranuclear inclusion body was formed in the neurones. They state that similar tissue changes occurred in the liver and kidney.

The particle size of encephalomyelitis virus in brain suspensions has been estimated at 500$\mu\mu$ by Krueger, Howitt and Zeilov.[16] They state that under like conditions of preparation and filtration it is of the same order of magnitude as the causal agent of poliomyelitis and apparently ten times the size of the hoof and mouth disease particle.

Material and Methods

Intensive microscopic study of the brains and spinal cords of eight horses affected by the virus of encephalomyelitis, either experimentally or by contagion, has yielded additional information as to the effects on the nervous system. As controls we have studied the brains and cords of three horses that died from sepsis with no clinical signs of encephalomyelitis, and the brain and cord of one normal horse that was killed by exsanguination. In addition the brains of two guinea pigs injected with filtered virus and killed with chloroform 3 and 4 days after successful inoculation were studied by the same methods. Also sections from various parts of the brain of a farmhand who died with symptoms similar to those of the equine encephalomyelitis, to which he had been exposed, were studied.

Portions of the brains and spinal cords of the experimental animals were fixed immediately after death in 10 per cent formalin, in formol-Zenker solution, or in Bouin's fluid. Material from the field animals was fixed in some cases shortly after and in other cases many hours after death, and was less satisfactory for histological study. The human brain was obtained at autopsy 12 hours after death and fixed in formalin.

Pieces from various parts of the nervous system were embedded in paraffin and sectioned at 5 microns. Sections were stained with Loeffler's methylene blue and basic fuchsin, or by Maximow's hematoxylin and eosin azur method. Prior to staining many of the sections were treated on the slide by immersion for 1 to 2 hours in Zenker's fluid and then washed with tincture of iodine for

removal of any precipitate of mercuric chloride. This treatment increased the stainability of the sections, especially of material fixed in formalin.

Other sections from a number of animals were subjected to tests for various pigments, since it was apparent early in the study that diffuse blood pigments in the tissues, and other pigments, especially in the large nerve cells of the brain stems of many of the horses, led to much confusion. It therefore became necessary to take into consideration the age of the animals and to test some of the material for blood pigments, lipochromes and melanin pigments. The tests applied were the alkaline alcohol treatment for blood pigments, the ether and chloroform solubility test for lipochromes and lipofuscin, the osmic acid color tests for lipochromes, and the osmic acid and silver nitrate tests for melanins. The sections subjected to these various procedures were afterward stained lightly with hematoxylin and eosin or with safranin. Through the courteous coöperation of Dr. Gordon H. Scott, of the department of cytology of the Washington University Medical School, the method of incineration on the slide was also used.

The tests for lipochromes leave much to be desired, since they could be applied only on material already subjected to the action of various fat solvents used in preparing paraffin sections. The combined results of the several tests, however, are worthy of some consideration and are described later. Lipofuscins are usually regarded as resistant to solution with alcohol after fixation other than directly with fat solvents. This fact, together with the yellow to yellowish green color of many of the inclusion granules, is considered indicative of lipofuscins.

Microscopic Examination

Sections of the brain and spinal cord of horses affected by the virus show the perivascular and leukocytic infiltration, in greater or less degree, already mentioned (Fig. 1). The cell types in the perivascular cuffs are lymphocytes, polymorphonuclear leukocytes and monocytes. In some of the horses many cells are phagocytic, containing numerous granules of brown pigment. Granule-laden phagocytes are also present within the blood vessels in a number of animals. The perivascular cuffs continue along the blood vessels for considerable distances and in some cases can be followed to the brain surface. Outside the layer of infiltrated cells there is a clear zone of varying width which in part, at least, represents shrinkage of the edematous brains, due to treatment with alcohol. The sections from most of the animals indicate considerable edema, especially in the brain stem and cortex. Diffuse blood pigments are present along the course of the blood vessels. These disappear on treatment with alkaline alcohol, as do the pigment granules in the phagocytic cells. The latter are accordingly also interpreted as blood pigments.

The control horses, three of which died from sepsis and one of which was killed, show no perivascular infiltration of cells. One of

the three dying from sepsis was not exsanguinated and shows an unusual amount of blood pigment in brain and cord. The two guinea pigs show no blood pigment, but both have perivascular infiltration, the one killed 4 days after inoculation in more marked degree than the other killed 3 days after being inoculated with virus. In the one human brain examined perivascular infiltration is also present, but little blood pigment is apparent. This brain was very edematous, as judged from the sections.

Many of the large nerve cells of the brain stem and spinal cord, and to a less extent in various parts of the cerebral cortex and cerebellum, show cytoplasmic inclusions in much larger numbers than in nerve cells of animals dying from other causes, as judged from the four control horses examined by us and from the large number of horses ranging in age from 2½ months to 30 years, reported by Kikuchi.[17] The inclusions consist of granule-like bodies which vary from 0.3 to 0.7 microns in diameter. Most of them are rounded but many of the larger ones are somewhat angular in form. In staining properties they show a wide range of variation which appears to depend in part upon the method of fixation and the state of the tissue when placed in fixing fluid. In material fixed in formol-Zenker's fluid immediately after death of the animal the eosin of the Maximow stain gives them a deep pink tint. In sections from blocks fixed in formalin or in Bouin's fluid they fail to stain with Maximow's technique unless previously treated on the slide with Zenker's fluid, and then they show much variablity in staining reaction. Usually, with this treatment, many of the granules take a pink tint, but many others remain nearly colorless or yellowish. In the same cell frequently are found granules ranging from pale yellow to pink in color. In much of the material yellowish green to green granules are present, especially after treatment with Zenker's fluid followed by iodine. This suggested that the granules are related to lipochromes and led to further tests for lipochromes and other pigments, as described in another part of this report in further detail.

The basic fuchsin stain colors the granules a brick red, irrespective of fixation. This stain was useful in locating groups of affected cells but was of little value otherwise, as compared with the other methods employed.

The distribution of affected cells in sections of material most

favorable for careful microscopic study is somewhat irregular. There are considerable areas of apparently normal cells, while at the margins of such areas are found groups of nerve cells showing inclusions and various stages of cell degeneration. In other sections considerable areas of affected cells occur, varying from nearly normal ones to cells in the last stages of disintegration. In general, nerve cells near blood vessels have progressed farthest in the degenerative process, suggesting that the destructive agent is brought by the vessels or along their sheaths.

Phagocytosis of Nerve Cells

Some of the injured nerve cells show the effects of marked activity of phagocytes (Figs. 3 and 7). Such cells are necrotic with shrunken eccentric nuclei whose nucleoplasm is basophilic and whose nuclear membrane usually has disappeared. The cytoplasm of these cells is shrunken and altered and Nissl bodies are markedly chromatolytic or absent. Inclusion bodies, however, are usually demonstrable. They have the same staining reaction and position as in slightly damaged cells. Phagocytes frequently may be seen burrowing into the surface of the nerve cells from all sides (Fig. 3) and affecting both perikaryon and cell processes. Extreme cases are encountered where only fragments of the nerve cells remain, surrounded by phagocytes. No phagocytes have been found deeply embedded within the nerve cells, as described by Da Fano and Ingleby [18] (see page 358), but the burrowing action of phagocytic cells is evident from Figure 3.

Many nerve cells, in which nuclei are still nearly or quite normal in appearance, show small vacuoles in the outer layers of their cytoplasm (Fig. 3). The vacuoles vary somewhat in size and distribution. Inclusion granules present in such cells lie in the deeper part of the cytoplasm. There is no apparent direct relation between vacuoles and granules.

Nuclei of Affected Nerve Cells

The fuchsinophile nuclear inclusions found by Joest [19] in the nerve cells of Borna's disease of horses, a disease that has many points of similarity to the horse encephalomyelitis of Haring and Meyer, naturally led to a careful search for intranuclear bodies in our material. Some cell nuclei in horse material (A147 and A252,

fixed immediately after death) contain rounded bodies of about the same size as those in the cytoplasm but, as a rule, of bluish color. Occasionally acidophilic granules appear in the nuclei. In animal A 41, a pregnant female 6 years old, the Maximow stain reveals reddish granules in the nuclei of many of the nerve cells. The granules are indistinct individually, but give a reddish tint to the nucleus. In this animal the cytoplasmic inclusions are small and are largely hidden by the Nissl substance. There was also but little perivascular infiltration in this animal, although clinically it had encephalomyelitis.

In guinea pigs injected with filtered virus numerous reddish granules are brought out by the Maximow method in many nerve cell nuclei, but most of the nuclei are clear and normal in appearance, even when the cells contain cytoplasmic inclusions. In these guinea pigs the cytoplasmic granules, as in horse A41, are small and more difficult to find than in most of the horse material. The greater part of the material examined, however, including the human, shows very little indication of anything but the normal chromatin granules in the nuclei by the methods employed. In view of the reported discovery by Syverton, Cox and Olitsky [15] of intranuclear inclusions in various animals subjected to the virus of equine encephalomyelitis, our failure to find nuclear inclusions constantly may be due to variations in the condition of the tissue when placed in fixing fluid, caused by differences of the time interval after death, and to other factors of technique which were not controllable in field animals and in human autopsy material. Many nuclei are found in various stages of destruction, with loss of nuclear membrane, and with acidophilic nucleoplasm, but only in markedly chromatolytic and otherwise necrotic appearing cells.

Cytoplasmic Inclusions

The inclusion bodies in the nerve cells occur, as a rule, as clusters of granules near the nucleus of the cell. Most frequently they are found at one side of the nucleus (Figs. 2, 3 and 5). Sometimes they occur in several clusters, which may be situated at opposite poles of the nucleus (Figs. 4 and 6). Not infrequently the granules are scattered throughout the cytoplasm without reference to the nucleus. In the larger nerve cells they have been encountered in clusters in

the dendritic processes at considerable distances from the perikaryon. Occasionally they occur as compact, well circumscribed masses of granules, either near the nucleus or elsewhere in the cytoplasm.

Such inclusions are found in nerve cells that show only slight indications of degeneration (Fig. 4), and they also are present in cells that are in the last stages of destruction and that are undergoing phagocytosis (Fig. 3). The hematoxylin-eosin azur method is particularly favorable for staining these granules and the Nissl substance at the same time, when the latter still remains. The Nissl granules take a grayish blue tint, in marked contrast to the unstained, or pink inclusions. In Figure 4 the large, flaky Nissl bodies are shown throughout the perikaryon, appearing in the photomicrograph as gray masses. The inclusion granules, somewhat masked by the Nissl bodies, are shown in the photomicrograph as smaller black dots at either pole of the nucleus. The nucleus itself has been but slightly affected in the cell shown in this figure. Another cell is shown in Figure 5, in which Nissl bodies still are present at the periphery of the perikaryon, but in which chromatolysis has progressed much farther. The nucleus also is pyknotic and eccentric in position. The inclusion granules, however, are altogether similar to those of Figure 2, and show more clearly in the photomicrograph because they are not partially hidden by Nissl bodies. The nerve cells shown in Figure 6 have degenerated markedly, but the inclusion bodies have the same appearance as in the other cells.

Many publications have appeared on intracellular and intranuclear inclusions in relation especially to virus disease. Findlay and Ludford [20] have reviewed this literature up to 1926 and Cowdry [21] has again covered the field up to 1927. Another survey is, therefore, unnecessary.

Hueck [22] has differentiated lipofuscin from other lipoid inclusions in nerve cells and other tissues. He states that there is considerable variation in the amount of lipofuscin in different types of nerve cells. Kikuchi,[17] as already noted, in 1928 described lipofuscin and other inclusions in the nerve cells of horses ranging widely in age. He considers lipofuscin to be a normal constituent of the nerve cells, but shows that it is more abundant in older animals. Bielschowsky,[23] agreeing in general that lipofuscin is present in normal nerve cells, holds that under certain pathological conditions there is a great in-

crease of this pigment. The nucleus in cells undergoing such pathological formation of pigments may remain well preserved for a long time but eventually succumbs, preliminary to destruction of the cell.

Da Fano [24, 25] has described minute bodies in herpetic encephalitis of rabbits. So far as can be judged from his descriptions and excellent figures these minute bodies are probably not identical with the cellular inclusions of the present account. Da Fano found bodies in various cells of the brain, including nerve cells. In our material the granules are found only in the nerve cells. There are also certain differences in the appearance of the granules themselves, as compared with the minute bodies of Da Fano. The latter are described [24] (see page 97) as a "single or double minute granule, roundish or slightly oval, surrounded by a clear halo; when in pairs the granules are so closely attached one to the other as to convey the impression of dumb-bell shapes; these are surrounded by a common and somewhat wider halo." Only occasional granules in our material show any sign of a halo and there are few if any double granules. In form the range in our material is from rounded to angular, as above described. Da Fano's minute bodies, furthermore, stain a deep purple-blue or purple-red with polychrome methylene blue and Giemsa's stains. Subsequently Da Fano [25] and Da Fano and Ingleby [18] reported minute bodies in epidemic encephalitis in man, but with negative results as respects their presence in nerve cells.

Cowdry and Nicholson [26] describe and figure inclusions in the nerve cells of rabbits subjected to herpetic virus, which in size and general characteristics are not unlike those here described. In staining reaction, however, the granules of these investigators were colored a deep blue by the Giemsa stain on air-dried films.

It is apparent from Table I that there are at least three, and probably four, kinds of pigment in some of the brains studied by us. The blood pigments, while confusing because of their wide diffusion in the sections, are easily eliminated by the alkaline alcohol treatment. The melanin pigments can usually be recognized without difficulty in hematoxylin and eosin sections, and on treatment with silver nitrate they show the blackening that is characteristic of this test. With osmic acid they assume a deeper brown coloration. They are not dissolved in ether and chloroform. In a normal horse, 12 years of age, such granules are present in considerable numbers in the

TABLE I

Results of Various Microchemical Tests Applied to the Cytoplasmic Inclusion Bodies

Material	Age	Brain part	Silver nitrate	Osmic acid	Ether and chloroform	2% Sodium hydroxide in 80% alcohol	Incineration at 600° C
Normal horse	*yrs.* 12	Medulla oblongata	Black granules				
		Midbrain	Black granules	Brown granules			
Horse No. A147	Aged	Various parts	Yellow granules	Dark brown granules	Pale to yellowish granules	Blood pigments dissolved, other granules present	Granules disappear save for a slight ash
Horse No. A252	16	Various parts	Yellow granules	Dark brown granules	Yellow granules	Blood pigment dissolved, pale granules in nerve cells	Granules disappear save for a slight ash
Horse No. 1683	Young	Midbrain	Some black granules	Brown granules	Granules faint to fairly distinct		
Horse No. 1684	Young	Midbrain	Yellow to black granules	Brown granules	Yellowish granules		
		Olfactory bulb	Diffuse precipitate		Pale granules		
Human No. 1513	43	Cerebral cortex	No blackening	Brown granules, vacuoles	Yellow to brown granules	No blood pigment, pale granules in nerve cells	
		Deep cerebellar nuclei	Some black granules	Yellow and brown granules	Brown granules	No blood pigment	
		Purkinje cells	No blackening	Light brown granules	Pale granules, some solution	No blood pigment	
		Hippocampus		Brown granules, some vacuoles	Brown pigment	No blood pigment, pale granules in nerve cells	

brain stem. Horses A147 and A252, although older than the normal horse, do not show any brown granules except after treatment with osmic acid. In sections treated with silver nitrate only yellowish granules are visible in the nerve cells. Treatment with ether and chloroform is followed by some indication of solution of some of the inclusions, but most of the pale to yellowish granules still remain. The poor solubility and the color of these inclusions point to lipofuscins. On incineration of thin unstained sections, mounted on the slide with distilled water only and subjected to a temperature of 600° C in an electric oven, the organic substance of the cells, including the granules, entirely disappears. Only a reddish ash suggesting iron is left in the position of the granules, according to Dr. Scott, to whose generous coöperation we owe the application and interpretation of this test. The younger horses, Nos. 1683 and 1684, show some melanin in the midbrain but very little if any elsewhere. Inclusion bodies are widely distributed, however, in this material, appearing as yellow granules after all the tests, save the osmic acid which turns them brown.

Sections from various parts of the human brain which had been fixed in formalin show a considerable range of reactions to the tests applied. Only in the deep cerebellar nuclei are there granules that give positive indications of melanin, but sections through the deep part of the midbrain were not available. Cerebral cortex, hippocampus and the Purkinje cells show yellow to brown granules, with some solution by ether and chloroform. The yellowish green to green color of granules observed in some of the horse material was also observed in some of the human nerve cells.

The results of the tests as to the nature of the inclusions point in the direction of lipochromes and lipofuscin. The variations in staining qualities appear to depend not only upon the stain employed and the preliminary treatment, but also upon changes that must take place within the granules themselves, as shown by the presence in many cells of granules that range from pale yellow to deep pink in color when treated by Maximow's eosin azur stain. There is, however, no apparent relation between the stage of destruction of the nerve cells containing them and the staining qualities of the granules, since in material fixed immediately after the death of the animals, *e.g.*, horses A147 and A252, nerve cells in various stages of necrosis are present in which no differences are apparent in the granules.

Summary and Conclusions

Histological and cytological study of the brain and spinal cord of horses, guinea pigs and humans subjected to the virus of equine encephalomyelitis shows characteristic pathological changes. The most constant feature is the perivascular infiltration already pointed out by Meyer and Haring. This is found in all horses affected by the virus, but in none of the controls. It is also present in the guinea pig and human brains that were affected by the virus.

There are suggestions of intranuclear inclusions in some of the nerve cells in several animals, but in our material this feature is too inconstant to permit of considering them as characteristic features of the affected cells.

There is considerable degeneration of Nissl substance in many nerve cells of virus-infected animals and also in the human brain. Nerve cells in various stages of necrosis are present, especially in the brain stem and spinal cord. Many nerve cells are in process of phagocytosis by leukocytes.

Cytoplasmic inclusions are present in many nerve cells of all the animals studied, which were affected by the virus, and in the human brain. Similar inclusions are found in smaller numbers in three horses that died from an unknown sepsis and also in a normal horse 12 years of age. The number of inclusions in the nerve cells of the virus-infected animals is considerably greater than in the control animals, and appears to be increased by the pathological conditions of the disease.

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DESCRIPTION OF PLATES

PLATE 97

FIG. 1A. Photomicrograph showing the perivascular infiltration following encephalomyelitis in the brain of a horse.

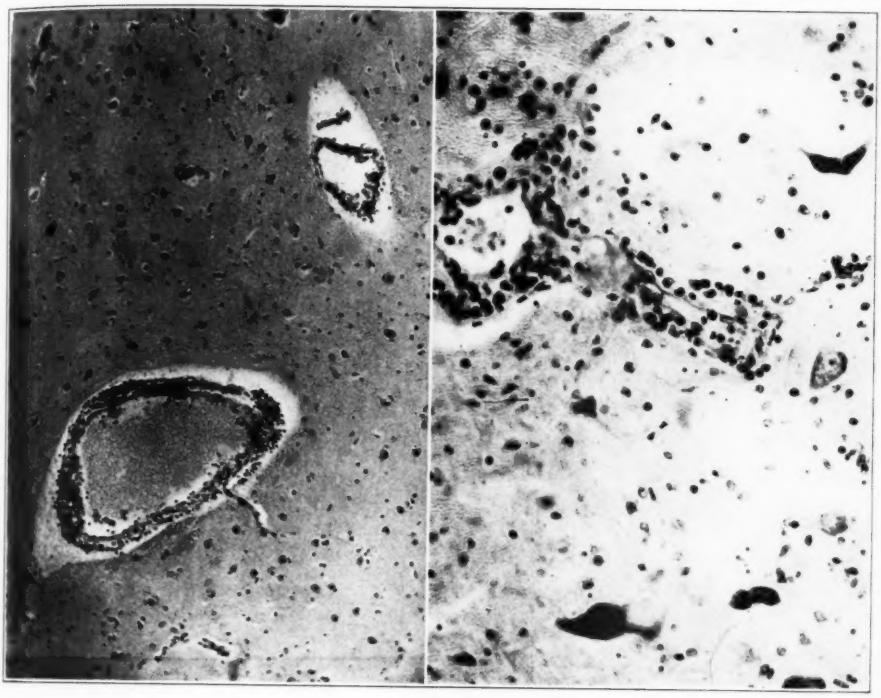
FIG. 1B. Photomicrograph with higher power showing perivascular infiltration and also nerve cells in various stages of necrosis.

FIG. 2. Brain stem of horse No. A147, showing nerve cell and small blood vessel with cytoplasmic inclusions in a necrotic cell and perivascular infiltration, respectively. Hematoxylin and eosin azur stain after Zenker fixation. Section 5 microns.

en = endothelium; In = cytoplasmic inclusions; N = Nissl bodies. × 820.

FIG. 3. Horse No. A147. Nerve cell surrounded by phagocytes. The cell is necrotic and also shows inclusions. Hematoxylin and eosin azur stain after Zenker fixation. Section 5 microns.

In = cytoplasmic inclusions; Nu = nucleolus; ph = phagocytes. × 820.



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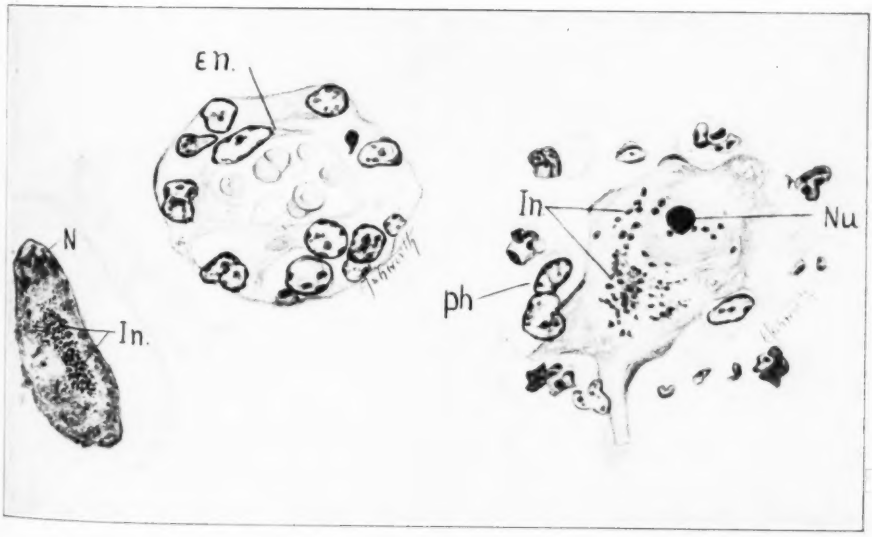


2 3

Larsell, Haring and Meyer Histological Changes in Central Nervous System

Plate 98

Fig. 4. Horse No. A147. Photomicrograph of nerve cell in brain stem showing Nissl bodies (grayish) and cytoplasmic inclusions (black). Hematoxylin and eosin azur stain after Zenker fixation. Section 5 microns.

In = cytoplasmic inclusions; M = precipitate of mercuric chloride; N = Nissl bodies; Nu = nucleolus.

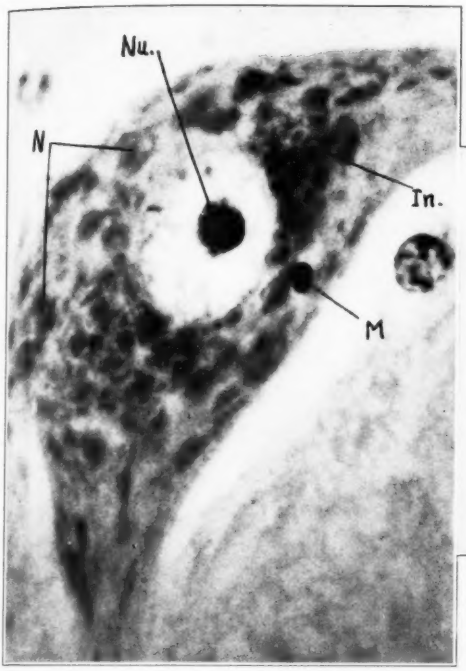
Fig. 5. Horse No. A147. Photomicrograph of nerve cell in later stage of destruction than that shown in Fig. 4. Nissl bodies are found only at the periphery of the cell and the nucleus is eccentric and shows considerable degeneration. Hematoxylin and eosin azur stain after Zenker fixation.

In = cytoplasmic inclusions; M = precipitate of mercuric chloride; N = Nissl bodies; Nu = nucleolus.

Fig. 6. Photomicrograph of nerve cells markedly degenerated with advanced chromatolysis. Hematoxylin-eosin azur stain after Zenker fixation. Section 5 microns.

In = cytoplasmic inclusions; M = precipitate of mercuric chloride; Nu = nucleolus.

Fig. 7. Brain stem of horse No. A252 showing a group of nerve cells undergoing phagocytosis. Hematoxylin and eosin azur stain after Bouin fixation. Section 10 microns.



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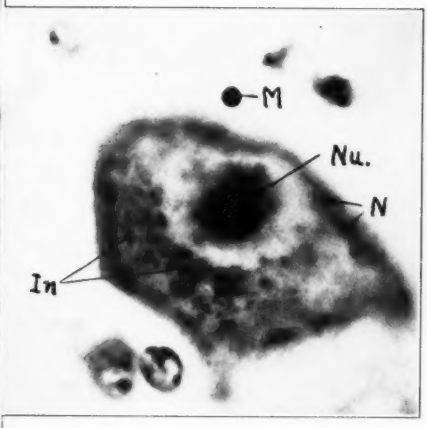

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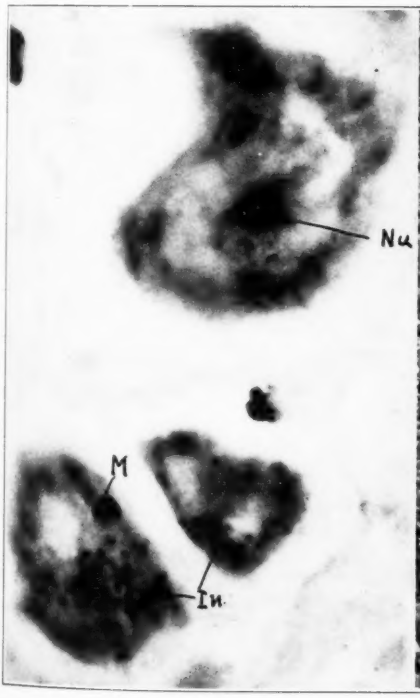

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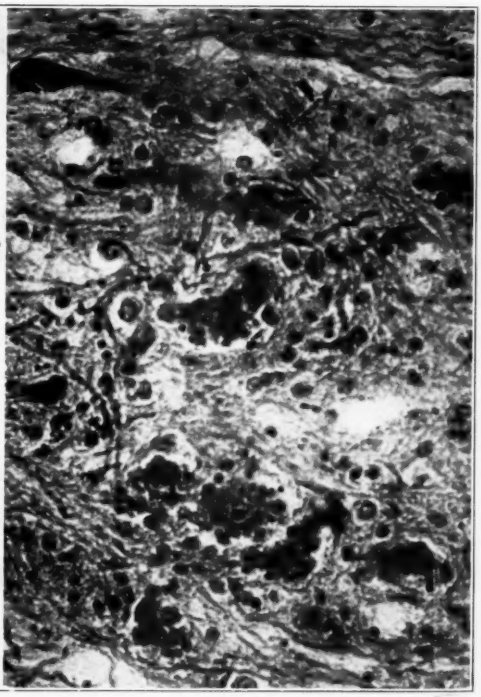

7

ANOMALIES OF THE INTERVENTRICULAR SEPTUM AND PULMONARY ORIFICE *

REPORT OF TWO CASES

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Malformations representing arrests at successive stages in the evolution of the developing heart have recently been observed in this laboratory in 2 cases. Each showed a defect in the interventricular septum which was associated with pulmonary atresia and dextroposition of the aorta in 1, and with stenosis of the pulmonary orifice in the other. The first case is of further interest in that it is one easily mistaken for truncus arteriosus communis persistens, and the second because it suggests the probable pathogenesis of the malformation in this case. Abbott's[1] review of 850 cases of cardiac malformations yielded 186 instances of basal defects of the interventricular septum complicating other anomalies. Among these 95 had pulmonary stenosis or atresia, and associated dextroposition of the aorta was encountered in 62 of the latter group. In view of the rarity of the condition these two cases seemed worthy of reporting.

CASE REPORTS

CASE 1. R. W., an 18 months old negro boy, was admitted to the New Haven Hospital on March 21, 1933, appearing acutely and gravely ill. He had been under observation in the Children's Community Center of New Haven and was known to have congenital heart disease and a spastic right-sided hemiplegia of unexplained origin. According to the mother this was her fifteenth child and all the others were living and well. The mother was 42 years of age. A Kahn test of the blood of both the father and mother was negative. The child, born at term on Oct. 17, 1931, following an uneventful pregnancy, was delivered spontaneously and weighed 4025 gm. The only unusual features noted at birth were "a collodion-like membrane" which peeled off in large pieces from the normal skin, and scant development of the eyebrows and eyelashes. On the 4th day after birth a rise in temperature to over 38° C developed and lasted for about a day and a half. On Oct. 28, 1931, a blue coloration of the lips and nail beds was noted and a double murmur was heard over the entire precordium and the left back. Roentgenographs showed an unusual cardiac silhouette and

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a marked enlargement of the left ventricle. The transverse diameter of the cardiac shadow measured about 6 cm., while the intrathoracic diameter at the level of the ninth rib was 10 cm. The mediastinal shadow was normal and there was no prominence of the shadow of the pulmonary artery. When discharged from the hospital the next day the infant weighed 3860 gm.

He was then followed in the Outpatient Clinic and was seen first on Nov. 2, 1931, a few days following his discharge from the hospital. At this time he appeared to be in good health, weighed 4040 gm., and was taking the breast and formula well. When next seen on Nov. 23, 1931, the child weighed 4440 gm. and had a scaly papular lesion on the cheeks, scalp and skin folds. A systolic blow was heard over the left infraclavicular region. A roentgenogram revealed practically no change in the configuration of the cardiac shadow. During the next 2 months the child developed satisfactorily. On Jan. 18, 1932, he weighed 5320 gm. A roentgenogram taken at that time showed a slightly enlarged cardiac silhouette with a transverse diameter of 7.1 cm. and an intrathoracic diameter of 12.5 cm. The pulmonary shadow was not enlarged. On Feb. 8, 1932, the child weighed 5620 gm., showed definite cyanosis of the tips and nails of the fingers and toes, and some dyspnea when in the sitting posture. No murmurs were heard over the heart. In the second interspace, just to the left of the sternum, the first sound was not audible. On March 15, 1932, the weight was 6500 gm. In addition to a moderate degree of cyanosis of the lips, tips of fingers and toes, a widening of the cranial sutures and of the frontal and occipital fontanelles was noted. The circumference of the head was 40.5 cm. When seen on April 19, 1932, the child had a cough and looked rather ill, but his sleep and appetite were not impaired. He weighed 6890 gm. and his temperature was 39° C. A roentgenogram taken on April 22, 1932, showed the lung fields to be clear. There was a marked enlargement of the left ventricle. The transverse diameter of the cardiac shadow measured 7.8 cm. and that of the chest 13.4 cm. He seemed to have recovered from this episode when seen 5 days later. On May 25, 1932, he weighed 7260 gm., and the circumference of his head was 42 cm. On June 8, 1932, the mother noticed a swelling of the right cheek but this seemed to have disappeared when the child was seen again 2 days later. On his next visit, Oct. 14, 1932, the weight was 7120 gm.

He was seen again on Feb. 1, 1933, at which time the mother stated that on the previous day the child became fretful and that he cried and ate and slept poorly. The next day he ate better, was drowsy and awake only for feedings. It was noticed that the child cried when he was picked up by the armpits and that his right arm was limp and "not used." On Feb. 3, 1933, he was admitted to the New Haven Hospital. At this time he appeared thin and small for his age, inactive and drowsy. He weighed 7010 gm. and measured 73.5 cm. in height. The systolic blood pressure was 85 and the diastolic 50 mm. Hg., the pulse rate was 150 per minute, and the temperature was 39° C. There was a slight tachypnea but no obvious respiratory distress. Slight cyanosis of the lips and nail beds, with some clubbing of the fingers and toes, was noted. The circumference of the head was 44.2 cm. The frontal fontanelle was large, open and did not bulge. The frontal bones were prominent. The eyes were kept turned toward the left. The pupils were equal, regular and reacted to light. The ocular fundi appeared normal. The cardiac area seemed not to be enlarged on percussion. A loud systolic murmur was heard over the precordium, was transmitted to the great vessels of the neck, and was heard all over the cranium. A systolic thrill was felt in the suprasternal notch. A roentgenogram disclosed an

enlargement and boot-shaped configuration of the cardiac shadow, as well as an increase in the angulation of the tracheal bifurcation. Both pulmonary fields were clear. The right arm and leg showed a flaccid paralysis. The erythrocyte count was about 6,880,000 with 57 per cent hemoglobin; the white blood cell count was 11,100, with 62 per cent polymorphonuclear leukocytes and 34 per cent lymphocytes on several occasions. The urine contained no albumin or sugar. Roentgenograms of the skull, pelvis and extremities were essentially negative. Roentgenoscopic examination of the esophagus with a barium meal showed no narrowing. A prominence of the shadow of the pulmonary artery was also noted. During the subsequent stay of 5 weeks in the hospital the right hemiparesis gradually improved and the child gained some weight. An electrocardiogram taken on March 3, 1933, showed a sino-auricular tachycardia and left axis deviation (new terminology). When transferred to the Children's Community Center on March 13, 1933, the child weighed 7600 gm. All neurological signs had disappeared, except that the right leg was held flexed at the knee but could be extended voluntarily.

Three days after discharge from the hospital the child again became irritable, took food poorly and in the next few days became progressively weaker. On March 17, 1933, he had a brief episode of spastic quadriplegia. On March 19 he had an elevation of temperature which ranged between 39.4° and 41.2° C. The next day a discoloration was noted along a vein running over the forehead and bridge of the nose. When readmitted to the New Haven Hospital on March 21, 1933, the child appeared acutely and gravely ill and difficult to arouse. He weighed 6900 gm. and his temperature was 37° C. The bluish coloration of the bridge of the nose and forehead was still present. The eyes deviated to the right, showed a fine horizontal nystagmus, and the pupils reacted but little to light. The eyegrounds were essentially normal. There was marked cyanosis of the lips. The gums were reddened. A convulsion was produced when an attempt was made to examine the throat. The lungs were clear. The heart was not enlarged to percussion, its rate was rapid and regular. A blowing systolic murmur was heard loudest over the pulmonic area. A murmur was faintly heard over the right parietal region. Cyanosis and clubbing of the fingers and toes was noted. There was a slight spasticity of all the extremities and the tendon reflexes were more hyperactive on the right than on the left. The erythrocyte count was 8,120,000 with 74 per cent hemoglobin; the white blood cell count was 28,000 with 85 per cent polymorphonuclear leukocytes and 13 per cent lymphocytes. The urine contained no acetone, sugar or albumin. A lumbar puncture on the next day showed clear cerebrospinal fluid. The temperature rose to 39.8° C but was lower the next day and then remained normal with occasional rises to 38° C. Feeding required recourse to gavage, and hypodermoclyses were frequently given. On April 3, 1933, signs of pneumonia set in and on April 5 the child died.

At autopsy, in addition to the anomalies in the heart and large vessels, the following changes were noted: slight clubbing of fingers and toes, large open frontal fontanelle, increased circumference (44 cm.) of the head, hypoplasia of the thymus and spleen, chronic passive congestion of the viscera, bilateral focal pneumonia, hemangiectases of the veins of the right Sylvian fissure, thrombi in the left

cerebral artery and its branches with multiple old and recent infarcts of the brain.

Description of the Heart: In situ the heart was not particularly enlarged, its transverse diameter was 8 cm., that of the chest 14 cm. The apex was markedly rounded and made up equally of both ventricles. From the conus arteriosus a single vessel arose which at first sight appeared to be a common trunk for the pulmonary artery and the aorta. The disposition of the inferior vena cava, the azygos vein and superior vena cava, the coronary sinus and its tributaries appeared normal.

The heart weighed about 70 gm. The right half was slightly larger than the left. The right atrium appeared normal. The atrioventricular orifice measured 6.5 cm. in circumference; the valve was not altered. The wall of the right ventricle was 7 mm. thick; the trabeculae carneae and the musculi papillares were well rounded. There was an opening in the septum ventriculorum in the region of the membranous septum, 1 cm. in diameter, forming a communication between the ventricles. The orifice of the arterial trunk measured 4 cm. in circumference; the valves appeared normal. The left coronary artery arose from the left sinus of Valsalva, the right from the anterior sinus. The subsequent course of each of these vessels appeared normal. In the ascending portion the transverse diameter of the aorta measured 2.5 cm. From the arch, the innominate artery, the left common carotid and subclavian arteries arose. The patent ductus arteriosus continued into the right and left branches of the pulmonary artery. Below their division the pulmonary artery continued with a gradually decreasing lumen to the heart where it attached blindly as a fibrous band to the left of the aorta. No further anomaly was noted in the rest of the aorta. The left atrium showed nothing unusual; the foramen ovale was closed except for a narrow slit measuring 2 by 8 mm. The atrioventricular orifice measured 5 cm. in circumference; the valve appeared normal. The wall of the left ventricle measured 7 mm. in thickness. The trabeculae carneae and the musculi papillares were well rounded. No opening was present at the usual site of the aortic orifice.

CASE 2. F. F., a 9 year old white boy, was brought in to the emergency room of the New Haven Hospital on June 7, 1933. He was dead upon arrival. The child had been under observation in the Outpatient Clinic for a congenital

heart condition. According to the parents he was one of eight children, the rest of whom were living and well. The child was born in 1923 by a normal spontaneous delivery at term after an uneventful pregnancy. He was a "blue baby."

When first seen in the Outpatient Clinic of the New Haven Hospital on July 29, 1926, the child appeared well developed and nourished. He became cyanotic when lying down and when crying. The heart was not enlarged to percussion, the rhythm was regular. A harsh systolic murmur, maximal in the third left interspace halfway between the sternum and the nipple, was audible over the whole precordium. A short systolic thrill was felt over the point of maximum intensity of the murmur. A diagnosis of congenital heart disease with interventricular septal defect was made. The patient returned to the Clinic on Feb. 13, 1928. During the interim he had developed difficulty in breathing, which became gasping. At times he was pale, at other times his lips and face were cyanotic. The left chest in the precordial region bulged slightly. The cardiac signs were the same as on the previous occasion. The pulse was 134 per minute and the temperature 39° C. His throat was injected and his tonsils were large. The next visit was on May 15, 1931. In the interval he had had increasing attacks of dyspnea and cyanosis upon exertion, which had necessitated a marked restriction of his activities. There was definite cyanosis of the ears, lips, tongue and extremities, and clubbing of the distal phalanges of the fingers and toes. The heart sounds were the same as on former occasions. Roentgenographic examination revealed no abnormalities in the size and shape of the heart. When seen again on Sept. 25, 1931, he had attacks of precordial pain, was markedly cyanotic and definite venous pulsation was noted. It was assumed that decompensation of the heart had commenced, and accordingly his activities were severely restricted. He was last seen in the Clinic on April 15, 1932, at which time he was deeply cyanotic. The systolic blood pressure was 94 and the diastolic 86 mm. Hg. On June 7, 1933, 30 minutes before being brought to the emergency room, he is said to have had an attack of dyspnea and cyanosis in which he stiffened out and cried for air. Five minutes before arrival he became limp and his breathing stopped. Both the cardiac pulsations and the respirations had ceased at the time of arrival.

At autopsy the body was well developed. A distinct bulging of the thorax was noted in the precordial region. There was a striking dusky cyanosis of the skin, mucous membranes and nail beds and a marked clubbing of the distal phalanges of the fingers and toes. All the viscera showed signs of marked chronic passive congestion.

Description of the Heart: The heart *in situ* appeared greatly enlarged. The rounded apex was composed equally of the right and left ventricles. The transverse diameter of the heart was 12.5 cm., while that of the thorax was 19.5 cm. There was a striking disproportion between the size of the aorta and pulmonary artery, the aorta having a diameter of about three times that of the pulmonary artery. The disposition of the inferior vena cava, the azygos vein and superior vena cava, the coronary sinus and its tributaries, appeared normal.

The right atrium was somewhat dilated. The atrioventricular orifice measured 11 cm. in circumference; the valve was thin and delicate, except for one firm translucent nodule 2 mm. in diameter situated on the line of closure of the anterior leaflet. The wall of the right ventricle measured 0.8 cm. The conus arteriosus was large and was directly continuous with the cavity of the right ventricle. At the base of the pulmonary valve it was 2.5 cm. in circumference. The pulmonary artery was small; it measured 1.5 cm. in circumference, as did also its right and left branches. The wall of the vessel was thin and presented a smooth, shiny intimal surface.

The pulmonary cusps were fused and markedly thickened. At the free margin the edges were rounded and beset with firm, pearly gray, roughened excrescences which measured 2 to 3 mm. in diameter. The fusion of the three cusps formed an inverted funnel with an aperture at the apex not more than 1 mm. in diameter. The left atrium was of usual size. The foramen ovale was closed. The atrioventricular orifice measured 7.5 cm. in circumference. The leaflets of the mitral valve were thin, delicate and well formed. The cavity of the left ventricle was increased in size and the columnae carneae were somewhat flattened. The wall measured 0.8 cm. in thickness. The myocardium was a homogeneous red-brown. At the base of the interventricular septum there was a circular defect, 1.5 cm. in diameter, involving chiefly the membranous portion. The defect began posteriorly at the middle portion of the posterior cusp of the aortic valve and ended anteriorly on a line with the junction of the right and left cusps. It was bounded below by the crescentic, rounded free edge of the interventricular septum. The aorta, a large thick-walled vessel, arose equally from both ventricles and was overriding the septal defect. The aortic orifice measured 5 cm. in circumference; its valves were thin and delicate. The right coronary artery arose from the right sinus of Valsalva, the left from the left sinus. The vessels pursued the usual course and distribution. The arch of the aorta was well formed and the vessels arose from it in the usual manner. Each of the two bronchial arteries noted measured 2 mm. in diameter. The ductus arteriosus was a thin fibrous cord connecting the pulmonary artery and the aorta.

Microscopic preparations of the pulmonary valve show the basal portion to be thin and composed of loose strands of connective tis-

sue. Toward the free margin it is thickened by numerous nodules of dense acellular hyalinized connective tissue. In several of these deposits of calcium are present. The connective tissue adjacent to the nodules is infiltrated by small and large mononuclear cells. Several thin-walled blood vessels are present in the valves adjoining the nodules.

The interstitial tissue of the myocardium is infiltrated with small mononuclear cells. In many regions these are grouped in small foci of 20 to 30 cells adjacent to blood vessels. In the right ventricle the cells are distributed diffusely throughout the myocardium. The myocardial fibers of this ventricle are increased in size and contain bizarre shaped nuclei.

Discussion

The first case is one easily mistaken for a truncus arteriosus communis persistens. Two cases of this condition, which is rather rare, have been reported from this laboratory, one by Zimmerman [2] and the other by Finley.[3] Recently, in an admirable review, Humphreys[4] presented the criteria for identification of this malformation. In our case the surest landmark of the common trunk — the presence of four semilunar cusps, with two coronary arteries arising from the sinuses of opposite cusps — was absent. Furthermore, careful dissection disclosed the vestige of the pulmonary artery attached to the heart left of the aorta as a fibrous band. The latter, obtaining a gradually increasing lumen, continued into the right and left branches of the pulmonary artery; the patent ductus arteriosus connected these branches with the aorta. The case was thus identified as one of interventricular septal defect, atresia of the pulmonary artery and dextroposition of the aorta.

Stenosis of the pulmonary orifice without defect in the interventricular septum is, according to Abbott, "a lesion purely valvular and of inflammatory origin." When, however, the stenosis is associated with a defect in the interventricular septum, it is a malformation due to arrest in development. In our second case there is a healed valvulitis and a chronic diffuse myocarditis associated with this malformation. Thus, the question arises whether the stenosis of the pulmonary orifice is the result of the valvulitis or is a developmental defect with the valvulitis superimposed. The presence of

the septal defect favors the latter view. The rôle of the inflammatory process in the development of the stenosis cannot be estimated, although at present it is the most prominent feature.

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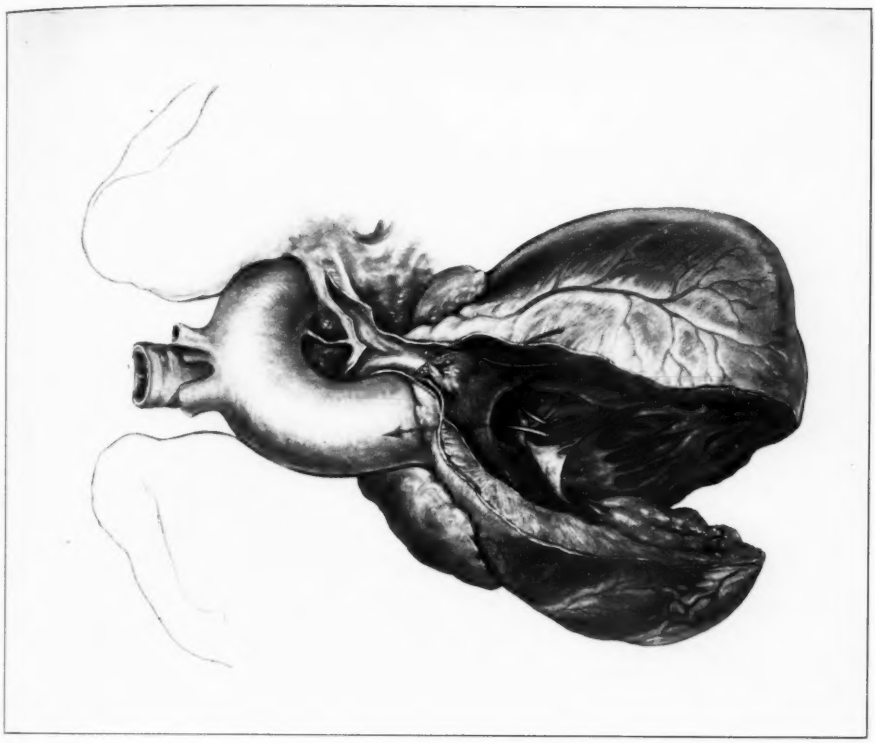
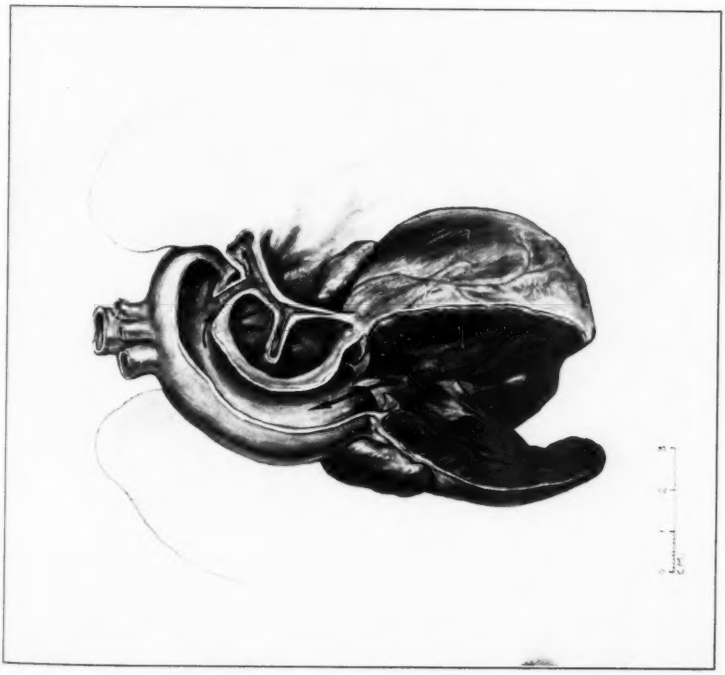
DESCRIPTION OF PLATE

PLATE 99

FIG. 1. Interventricular septal defect, atresia of the pulmonary artery and dextroposition of the aorta in an 18 months old negro boy. From the conus arteriosus a single vessel, the aorta, ascends to form the arch from which the arteria anonyma, the left common carotid and subclavian arteries arise. Attached to the heart, left of the aorta, is a fibrous band which, obtaining a gradually increasing lumen, continues into the right and left branches of the pulmonary artery. The patent ductus arteriosus connects these branches with the aorta.

FIG. 2. Interventricular septal defect and stenosis of the pulmonary orifice in a 9 year old boy. The caliber of the pulmonary artery compared with that of the aorta is small. The semilunar valves are thickened and fused, narrowing the orifice.





THROMBOPENIC PURPURA ASSOCIATED WITH CARCINOMA OF THE STOMACH WITH EXTENSIVE METASTASES *

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Carcinoma is practically always associated with a normal or an increased number of platelets in the peripheral blood.[1, 2] Perl [3] has recently reported values at the lower limits of normal in the majority of the cases in her series. From time to time various investigators [3-13] have reported exceptions to this general rule. Particularly uncommon is the association of thrombopenia with carcinoma of the stomach.[4, 5, 7, 8, 11, 13] In addition to the cases in which there was a definite thrombopenia, Ellermann [14] has described a case of carcinoma of the stomach with bone marrow metastases associated with ecchymoses. No estimation of the number of platelets in the peripheral blood was made by him. In the majority of the cases of carcinoma of the stomach with thrombopenia there have been extensive metastases to the bone marrow. Because of the rareness of the finding of thrombopenia as a complication of carcinoma of the stomach the present case, in which there were thrombopenia and extensive bone marrow metastases, is reported.

REPORT OF CASE

Clinical History: C. B., Unit No. 73002, 43 years of age, a male carpenter, was admitted to the Rochester Municipal Hospital on March 22, 1933, complaining of "stomach trouble." He stated that for 6 years he had had gaseous distention, belching of gas, sour eructations, and burning in the epigastrium and in the right abdomen. These symptoms occurred ½ to 2 hours after eating and were relieved by vomiting or by soda, but not by food. He had vomited frequently and, on occasions, had noted food eaten 1 or 2 days previously in the vomitus. His appetite was poor. For 2 weeks he had been troubled with a severe constant pain in the lumbar region. This pain radiated to the hips, down the thighs and into the upper back. He had felt weak for 10 days. Three days before admission, and again on the folllowing day, he vomited coffee ground material and passed semiliquid black stools. He had lost 30 pounds in weight in 4 years. He had noted pallor for several months, and also had noted that he

* Received for publication November 2, 1933.

bled easily after shaving. There was no history of petechiae, or epistaxis, and there had been no gross hematuria. The past history was unimportant. His father had had frequent severe epistaxis and had bled for 4 to 5 hours once following the extraction of a tooth.

Physical Examination: The patient appeared chronically ill. The skin and mucous membranes were pale. There was moderate epigastric tenderness associated with some muscle resistance. The liver was palpable just below the costal margin. No other findings of interest were observed on admission, but on the next day many petechiae were present on the lower extremities.

Laboratory Data: Table I shows the principal morphological blood findings during the period of observation. Repeated examinations of fixed blood smears stained with Wright's stain revealed slight achromia. The red blood cells showed slight variations in size, both microcytes and macrocytes being present and the average size being normal. No poikilocytosis was present. Occasionally nucleated red blood cells were found. In one preparation 8 normoblasts were seen while counting 200 white blood cells. The platelets were markedly diminished, averaging about 1 to 2 per oil immersion field. The coagulation time (test tube method) was 12 to 16 minutes. The clot retraction was good. The clot showed normal elasticity on one occasion and on two other occasions it was friable. The bleeding time varied between 10½ and 29 minutes. The icterus index was 6. Numerous urinalyses revealed no noteworthy abnormality. A small amount of albumin was present during the first week. After this the albuminuria remained about the same but casts were constantly present in varying numbers. On only one occasion was a rare red blood cell seen. Numerous clumps of white blood cells were found in one specimen. Repeated examinations of the stools showed the presence of blood during the first 12 days. No blood appeared after this time. The Wassermann reaction on the blood was negative.

Roentgenographs of the dorsal and lumbar vertebrae showed no metastatic lesions. A gastro-intestinal series revealed findings that were interpreted as carcinoma of the pyloric region of the stomach with high-grade obstruction.

Course in the Hospital: Two days after admission the patient vomited a large quantity of muddy, grayish material, which gave a strongly positive guaiac test. There was no further vomiting. Lumbosacral pain was one of the most troublesome symptoms, but it disappeared 20 days after he was first seen. The gastro-intestinal symptoms were partly alleviated by a Sippy diet. He was given large doses of iron in the form of Blaud's pills and iron and ammonium citrate, without any effect on the blood picture. Twenty-five days after admission he suddenly became dyspneic and cyanotic in the morning and died that afternoon. Permission for autopsy was obtained.

Postmortem Examination

External examination of the body and of the serous cavities reveals no findings of importance. The heart shows evidence of mild chronic tricuspid and subacute mitral endocarditis. The spleen, pancreas, adrenals, kidneys, pelvic organs, vascular system, neck organs and skeletal system show no noteworthy abnormalities.

TABLE I

Data on Blood Findings During Period of Observation

Date	Red blood cells	Hemoglobin	Reticulocytes	White blood cells	Differential count								
					Basophiles	Eosinophiles	Myelocytes	Juveniles	"Stabs"	Segmented forms	Lymphocytes	Monocytes	Degenerated forms
	per cmm.	*gm. 100 cc.*	*per cent*	*per cmm.*									
3/22/33	3,500,000	9.0	...	10,700	0.5	2.0	...	...	...	69.0	19.5	7.0	2.0
3/23/33	3,680,000	9.2	1.1	11,700	0.5	...	...	...	...	71.0	15.0	12.0	1.5
3/26/33	2,650,000	7.0	...	8,400	...	...	8.0	...	...	78.0	10.0	4.0	...
3/28/33	2,500,000	6.0	...	6,500	...	...	...	...	...	...	...	...	...
3/29/33	2,770,000	6.8	...	...	...	...	...	...	...	...	...	...	...
3/30/33	2,660,000	6.2	...	6,200	...	...	...	...	...	...	...	...	...
4/2/33*	2,930,000	8.8	...	...	1.0	3.0	7.0	1.0	7.0	54.0	23.0	4.0	...
4/3/33	2,830,000	7.4	2.0	...	...	...	...	...	...	...	...	...	...
4/4/33	2,910,000	7.7	...	...	...	...	...	...	...	...	...	...	...
4/5/33*	3,400,000	8.2	...	10,550	...	...	11.0	3.0	11.0	54.0	16.0	5.0	...
4/6/33	3,200,000	7.7	1.6	6,100	...	0.5	7.0	...	...	69.5	17.0	6.0	...
4/7/33	...	...	1.9	...	...	...	...	...	...	...	...	...	...
4/8/33	...	...	3.6	...	...	...	...	...	...	...	...	...	...
4/10/33*	...	...	1.4	7,300	...	0.5	6.5	3.5	19.5	56.5	11.5	2.0	...
4/11/33	2,900,000	7.9	1.7	6,000	1.0	...	15.0	...	...	65.0	14.0	5.0	...
4/12/33	...	...	2.1	...	...	...	...	...	...	...	...	...	...
4/13/33	...	...	1.7	...	...	...	...	...	...	...	...	...	...
4/14/33*	...	...	...	8,950	1.0	1.0	7.0	4.0	17.0	52.0	14.0	2.0	2.0

* Schilling differential counts done at this time. In all other counts all neutrophiles were classified as segmented forms.

Lungs: The left lung weighs 625 gm., the right lung 700 gm. The pleural surface of the right lung is covered with fibrous adhesions, that of the left is smooth and glistening. They are mottled red and black, voluminous and somewhat heavy, crepitant to spongy in consistence, and along the margins are distended alveoli. The hilum nodes are small and deeply pigmented. The bronchial mucosa is bright red and injected. The pulmonary vessels are elastic and show no thickening. At the apex of each lung is a small puckered scar. Sections through the lungs reveal bright red, glistening surfaces from which fluid can easily be expressed. There is no evidence of consolidation.

Gastro-Intestinal Tract: The mucosa of the esophagus is smooth and velvety. The stomach contains about 500 cc. of light brown, fluid material, and at the pyloric region is a firm, annular mass, involving the walls for a distance of about 2 cm. above the ring. In one area this mass is ulcerated and presents a gray, necrotic crater. Section through the mass reveals a firm, white, fibrous, glistening surface. This tissue has invaded the peritoneum about the pylorus and several firm white lymph nodes of the same consistence are noted in this region.

Liver: Weight 2150 gm. The organ is large, dark brown, and over the surface of all lobes are small, rounded gray areas which are depressed below the surface. These vary in size from 2 mm. to 1 cm. in diameter, and are also visible on cut section. Many of them show small areas of hemorrhage and a few are surrounded by a hyperemic zone. The remaining liver tissue appears normal. The lobulation is regular and distinct.

Gall-Bladder: About 50 cc. of viscid bile is present in the gall-bladder. The wall is not thickened and the mucosa is smooth, the ducts patent.

Lymphatic System: In the retroperitoneal region are several large grayish glands which are firm and on cut section present a uniform gray surface.

Bone Marrow: The shaft in the region of the middle third of the femur appears thickened. The marrow is quite firm and on pressure holds its shape. Bone trabeculae are conspicuous in the marrow cavity. There is only a slight amount of the red element present, the greater portion being fat. The marrow of the vertebrae is red and appears somewhat hyperplastic. Section of the rib shows the

marrow cavity to be nearly empty, showing no hyperplasia. The marrow cavity of the sternum contains many bone trabeculae. The marrow here is also firm and shows only slight hyperplasia.

Anatomical Diagnoses: Carcinoma of the stomach with metastases to the liver, lungs, adrenals, bone marrow, and lymph glands; pulmonary congestion and edema; acute bronchitis; subacute mitral and chronic tricuspid endocarditis; chronic cystitis; emphysema; healed apical tuberculosis; chronic pleuritis.

Microscopic Examination

Heart: The cardiac fibers appear small and there is a small amount of yellow pigment at the nuclear poles. Sections show no scars.

Lungs: The alveolar walls are congested and in many areas their lumens are filled with fibrin and fluid. Large endothelial phagocytes and heart failure cells are conspicuous. There are several small areas of infarction showing uniform infiltration with red cells and destruction of alveolar architecture. The most striking feature is the distribution of tumor cells. The lymphatics about the bronchi and vessels, and the capillaries of the alveolar walls, are in most areas filled with tumor cells. Also, the smaller vessels and the lymphatics of the pleura show a similar condition. The cells are large with irregular, pale cytoplasm. The nuclei are round or oval with prominent mitoses. There are no areas in which nodules of tumor have eroded lung tissue. A few small vessels contain thrombi, apparently invaded by tumor. There is no increase in fibrous tissue.

Spleen: The pulp is considerably congested. The malpighian bodies are small and there is conspicuous thickening of the central vessels. No tumor cells are present in the section.

Gastro-Intestinal Tract: Sections of stomach show epithelium that has undergone postmortem change and is eroded. In one portion it becomes atypical, presents a diffuse distribution and the cells are heaped up. The wall in this portion is thick and the muscle is almost entirely replaced by connective tissue. Tumor cells have invaded this area but are in small clusters and infrequent. They are surrounded in all instances by very dense fibrous tissue. The cells have round or oval, deep staining nuclei, rich in mitoses, and are quite small. Tumor cells are present on the serous surface, and there is evidence of chronic inflammation, with many clusters of round

cells. In one area the mucosa is definitely eroded, infiltrated with neutrophils and covered with cellular débris.

Liver: There are clusters of tumor cells that have stimulated a marked growth of connective tissue, almost completely obliterating the normal architecture. In these areas the proportion of connective tissue is far greater than that of tumor. The surrounding liver cells are atrophic and contain yellow pigment. Throughout the entire section there are tumor cells within the sinuses and in many lobules, completely filling the central veins. The cells are similar to those noted in the lungs and stomach. This latter type of tumor infiltration has produced no connective tissue formation. The distribution is remarkably like that seen in the leukemias. Many neutrophils are seen throughout the sinuses.

Adrenals: There is invasion of the medulla with tumor cells. They have not entirely destroyed the medullary tissue, are not abundant, and have stimulated no connective tissue formation.

Kidneys: A few small scars are present beneath the capsule, infiltrated with round cells and involving hyalinized glomeruli. The tubular epithelium is somewhat swollen and granular.

Bladder: The mucosa is eroded, and the submucosa is somewhat thickened and infiltrated with round cells. The muscle fibers are enlarged.

Lymphatic System: The mesenteric glands are almost entirely replaced by tumor cells.

Thyroid: Shows the usual adenomatous change. There is a slight amount of colloid in the acinar lumens.

Bone Marrow: The femoral marrow cavity shows a large amount of fibrosis. The connective tissue is quite dense and cellular, and spicules of bone are prominent. They are dense and show no evidence of destruction by osteoclasts. Throughout the marrow cavity are clusters of tumor cells, similar to those described in the stomach, which lie between the bony spicules and have not eroded the bone. A section through the shaft of the femur shows dense bone formation with clusters of eosinophilic and neutrophilic myelocytes, as well as the mature polymorphonuclear type. Erythroblasts and mature red cells are conspicuous. Normal appearing megakaryocytes are present but are probably diminished in total numbers. The sternal marrow shows a similar picture but the fibrosis is more marked and fewer tumor cells are noted. There is much less evidence of blood

cell formation in this region and the bony spicules are more prominent. Section of the vertebra shows an extensive infiltration of tumor cells without erosion of bone. There is moderate hyperplasia, possibly more marked than in the femur, of both red and white cell progenitors. Normal appearing megakaryocytes are numerous. Figure 1 shows a typical section of the vertebral marrow. Clusters of tumor cells are interspersed with normal bone marrow.

Discussion

So far as the blood picture is concerned, there is only one finding that is unusual — the marked diminution in the number of the platelets. The exact mechanism responsible for this cannot be given. It would seem that the presence of large numbers of tumor cells in the bone marrow was the probable cause of the thrombopenia. However, studies of the bone marrow sections reveal an approximately normal number of morphologically normal megakaryocytes. Nevertheless, the presence of these megakaryocytes does not prove that these cells were functionally normal. Increased peripheral destruction or loss of platelets cannot be excluded.

The question naturally arises as to the relation of the thrombopenia to the hemorrhagic diathesis. It seems to us that the connection is most probably a close one, although it would be impossible to say that the bleeding phenomena were not dependent, at least in part, on changes that may have occurred in the permeability of the capillary walls, as is thought by many to be the case in idiopathic thrombopenic purpura.

The presence of large numbers of early cells of the myeloid series and of numerous nucleated red blood cells is not unusual, and has been commented upon previously.[2, 4, 5, 6, 10, 11] These findings should be emphasized, however, as they may aid in differentiating idiopathic thrombopenic purpura from the symptomatic form in cases where the presence of a malignant tumor is not proved. It is unusual to find large numbers of nucleated red blood cells and many early myeloid cells in idiopathic thrombopenic purpura, although they may be found after recent massive hemorrhage.

The value of studies of the sternal marrow in such individuals cannot be too strongly emphasized. Had we been able to obtain a sternal marrow biopsy from this patient during life, a positive

diagnosis could have been made. This procedure was planned for this patient but his sudden death prevented its being done.

Summary

The clinical and postmortem findings in a patient who had thrombopenia associated with carcinoma of the stomach with extensive metastases are reported.

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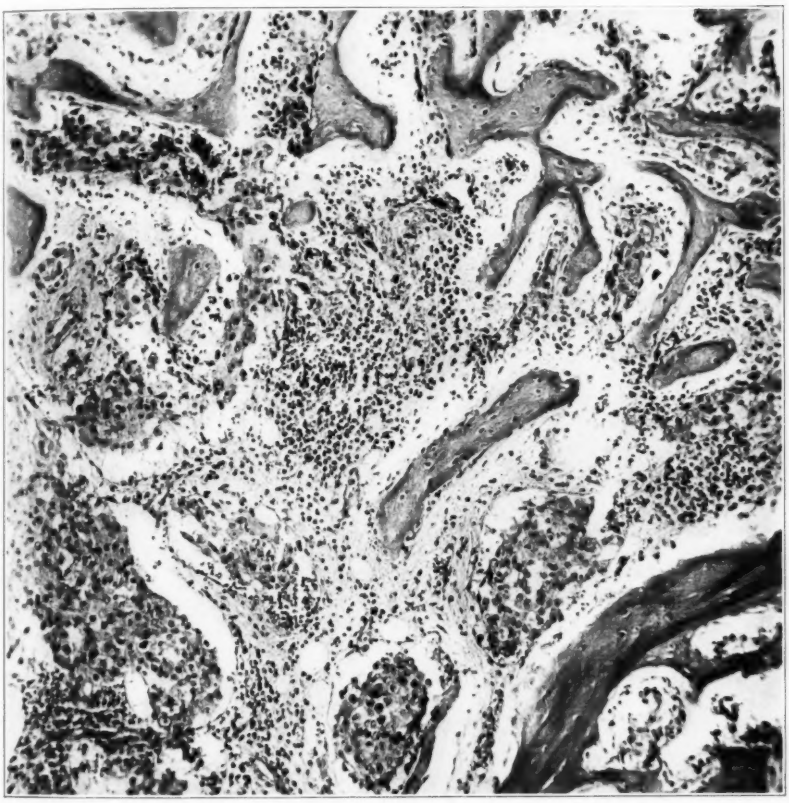
DESCRIPTION OF PLATE

Plate 100

Fig. 1. A typical section from the vertebral marrow. Note the presence of clusters of tumor cells interspersed in normal appearing bone marrow. × 115.



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TRACHEO-ESOPHAGEAL FISTULA OF SYPHILITIC ORIGIN *

REPORT OF A CASE

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Syphilis, as the etiological factor of tracheo-esophageal fistula, is uncommon. In 1899 Sirot [1] collected 143 cases, of which 68 were due to neoplasm, 11 to foreign bodies, 2 to pressure of a tracheal cannula, and 4 of which followed a cicatrix, 17 inflammatory lesions such as tuberculosis, 3 perforation of an ulcer, and 1 each to aneu-

TABLE I

Data on Instances of Tracheo-Esophageal Fistula of Syphilitic Origin Collected from the Literature

Author	Sex	Age	Site of tracheal lesion
		yrs.	
Berger (quoted by Conner [3])	M	33	Upper 1/3
von Navratil [9]	M	28	Upper 1/3
Krassnigg [10]	M	53	Middle 1/3
Schütze [11]	F	42	Lower 1/3
Gerber [12]	M	65	Lower 1/3
Levy [13]	F	24	Lower 1/3
Sonntag [14]	M	34	Lower 1/3
Beeler [4]	M	58	Lower 1/3
Dufour [15]	F	38	Not given
Moritz [2]	F	36	Not given
Basch [16]	..	..	Not given
Curschman (quoted by von Fraenkel [8])	F	41	Not given

rysm and trophic disturbances. In 36 cases the cause was undetermined. Only 1 of these, a case reported by Moritz,[2] was of definite syphilitic origin. In 1903 Conner [3] reported 128 instances of syphilis of the tracheobronchial tree. Two of these tracheal ulcers were followed by a perforation into the esophagus. We were able to collect only 12 instances of tracheo-esophageal fistula of syphilitic origin from the literature, and in 1 of these, Beeler's case,[4]

* Received for publication November 4, 1933.

there is some doubt as to the correctness of the diagnosis. The author himself raises the question. Table I is a brief résumé of the 12 collected cases. The case which follows is an addition to that list.

Report of Case

Clinical History: The patient, a negress aged 42 years, was admitted to the Jefferson Medical College Hospital on Aug. 15, 1932. For 10 days she had had great difficulty in swallowing both solid and liquid food. This alarming symptom developed very suddenly and was associated with a violent paroxysm of coughing and considerable expectoration. Prior to this event, the patient had had some cough but no expectoration. For some time she had noticed some dyspnea which became progressively worse. The dysphagia had been so intense that not only the swallowing of solids and liquids, but also attempts to swallow saliva induced a violent fit of coughing and choking. Expectoration was profuse, purulent, foul smelling, bloody, and at times contained foodstuffs such as milk, which was readily recognized. There was no pain in the throat or chest. Since the onset of illness the patient had lost weight rapidly.

The previous medical history contained little of interest. The patient had had five children. There was no history of miscarriages. She had had three consorts. The personal and familial record of tuberculosis and malignant disease was negative. There was no history of swallowing escharotics or of the presence of a foreign body in the trachea or esophagus.

Physical Examination: The patient was an extremely emaciated adult negress. The face was thin and pinched, the eyes sunken, the lips dry and parched. The skin was dry and harsh. There was remarkable freedom from dyspnea when the patient was quiet. She was not hoarse.

The eyes reacted to light and accommodation and the pupils were equal.

The mouth was rather dirty. Many of the teeth were decayed; the gums and gingival margins were the seat of a low grade, suppurative process. The tongue was markedly coated. The tonsils were not diseased and there were no paralyses affecting the tongue or soft palate.

The mucosa of the larynx was pale. Motility of the arytenoid cartilage and the vocal cords was not impaired. The pyriform sinus contained a small amount of saliva. No gross lesions were found in the larynx. Examination of the neck was essentially negative. The chest was long and flat and because the patient had lost so much weight, the ribs stood out prominently. There was no asymmetry of the thorax and the expansion was equal on both sides. Vocal resonance and tactile fremitus were not abnormal and percussion elicited no areas of impairment. Breath sounds were vesicular in character and moist râles were present at both bases of the lungs.

The heart was not enlarged and auscultation elicited no adventitious sounds.

The abdomen was rather markedly distended, the distention being chiefly confined to the upper half. The liver and spleen were not palpable. No pain, tenderness, rigidity or signs of fluid were present.

An examination of the extremities, including the testing of the reflexes, revealed nothing of importance.

The superficial lymph nodes, including the epitrochlear nodes, were palpable, small and hard.

The temperature was 99 F, the pulse rate 140 and the respiratory rate 28 per minute, on admission.

Special tests were made to determine the nature of the disability in swallowing. The patient was placed successively in the recumbent position, sitting position, on the right side and left side, and lying on the abdomen. While in each of these positions she was given a few sips of water to swallow. In the recumbent position she was able to swallow the water without difficulty. In the other positions the act of swallowing was accompanied by a violent paroxysm of coughing and expectoration of the swallowed fluid. A very brief but definite time intervened between the swallowing of the water and the onset of the cough.

The roentgenological report is as follows: "There is no evidence of a pulmonary lesion. The heart and diaphragm shadows are normal. We were unable to get films of the patient's swallowing function because every time we had her drink barium she had to cough. The barium gets down the pharynx, but instead of going down the esophagus, seems to be displaced so that it passes into the trachea, causing the patient to cough. However, I believe a very small amount of barium did get into the stomach. The area of obstruction seems to be at the beginning of the esophagus."

The laboratory reported a 4+ reaction of the blood when tested by the Wassermann and Kahn methods. The blood count was as follows: hemoglobin 65 per cent, erythrocyte count 4,000,000, and leukocyte count 14,800. The sputum was repeatedly examined for tubercle bacilli but none was found. The results of other laboratory tests were unimportant.

Esophagoscopy was performed and the report is as follows: "The pus was traced down to an opening in the esophagus which is partially covered by a necrotic flap. It is situated 21 cm. from the upper teeth, in the left quadrant of the esophagus. Air was forced through this fistula at each expiration and the necrotic flap blew in and out with the air current. The fistula is approximately 2 cm. in diameter. A Rehfuss tube was inserted into the stomach by the aid of the esophagoscope for feeding purposes." (See Fig. 1.)

The patient was given antiluetic treatment but became progressively worse. The temperature mounted to 105° F and symptoms of bronchopneumonia supervened. Death took place 5 days after admission.

Postmortem Examination

The body is that of an extremely emaciated adult colored female, aged 42 years. The lips are dry, the tongue coated, the teeth partially decayed and about the gums there is some pyorrhea. Superficial lymph nodes of the neck, axilla, groin and epitrochlears are small and hard.

Pleural, pericardial and peritoneal membranes are smooth and glistening and the respective cavities contain no excess of fluid. There is a mass filling the entire superior mediastinum. The thoracic contents are removed *en masse*.

Superior Mediastinum and Contents: The lymph nodes of the superior mediastinum are enlarged and firm, and are embedded in a

firm fibrous mass that also binds the other structures, vessels, trachea and esophagus together. On section the lymph nodes are yellowish white, firm, homogeneous, slightly granular and opaque. On the inferior aspect of the arch of the aorta there is a small aneurysmal pouch 1 cm. in depth. The endothelium of the vessel in this pouch is scarred, puckered and white, making a distinct contrast beside the yellow color of the intima of the aorta.

Trachea: The trachea is adherent to the esophagus and the other structures of the mediastinum. On section a large, circular, ragged ulcer measuring 6 cm. in its greatest diameter is found (Fig. 2). This ulcer is on the left posteriolateral aspect, reaching from the level of the first to the eighth tracheal rings. The margins of the ulcer are irregular, and slope down to the base in a step-like manner. The floor of the ulcer is ragged, dull, green and friable. The tracheal cartilages in the ulcerated area have completely sloughed and there is no vestige of them remaining. In the center of the ulcer there is a perforation 2 cm. in diameter, which communicates with the esophagus. The tracheal side of the perforation is surrounded by ragged friable shreds of tissue. The firm adhesions between the two organs have prevented leakage of contents into the mediastinal space.

Esophagus: At the upper end of the esophagus there is an oval-shaped perforation 2 cm. in diameter which communicates with the trachea (Fig. 3). The margin of the stoma is slightly elevated, fairly firm, quite red and clean cut. The remainder of the esophagus presents no gross pathological lesions.

Lungs: Both lungs are edematous and throughout there are numerous, small, red, firm areas of consolidation that fade imperceptibly into the sound tissue. The mucosa of the bronchi is swollen and red and the bronchi are filled with yellow purulent material. No gross lesions of tuberculosis are present.

Heart: Weight 470 gm., measurements 15 by 9 by 6 cm. The organ is of moderate size and there is slight hypertrophy of the left ventricle, otherwise it has no demonstrable pathological lesions. The aorta has no lesions of consequence except the small aneurysm described above.

Diaphragm: This appears to be without pathological change.

Spleen: Weight 150 gm., measurements 14 by 8 by 3 cm. No gross lesions are found, but considerable blood oozes from the cut surface.

Liver: Weight 1850 gm., measurements 28 by 24 by 7 cm. This organ is fairly large, flabby and chamois-colored. On section the parenchyma sinks beneath the cut edge and blood oozes from the cut surface. The left lobe gives the impression of being larger than usual. In the lower anterior portion there is a sharply circumscribed, irregularly shaped tumor measuring 7 cm. in diameter (Fig. 4). Its surface protrudes slightly above that of the liver and the mesentery is adherent to it. The tumor is pale yellow, firm and nodular. On section it is pale yellow, firm, homogeneous, dull, opaque and slightly granular. At its center there is some necrosis with softening.

Kidneys: The right kidney weighs 150 gm. and measures 11 by 6 by 4 cm.; the left weighs 120 gm. and measures 10 by 6 by 4 cm. They are somewhat red and on section considerable dark red blood oozes from the cut surface.

The following organs show no gross pathological lesions: *gall-bladder* and *biliary ducts, adrenals, pancreas, ureters, bladder, stomach, small* and *large intestine,* and the *abdominal lymph nodes.*

Uterus: A few small, subserous myomas are present which measure about 0.5 cm. in diameter. The *ovaries* are fibrosed. The *fallopian tubes* are dilated, tortuous and adherent to the ovaries. They contain no pus or other fluid.

Microscopic Examination

The lesions that are of particular interest histologically are those of the trachea, thoracic lymph nodes and liver. Microscopically the ulcer of the trachea consists of a central necrotic area and a marginal cellular zone. In the marginal zone there is a decided increase in fibrous tissue and capillaries. The tissue, particularly about the vessels, is infiltrated with cells of inflammatory origin, with a preponderance of fibroblasts, epithelioid cells, lymphocytes and other mononuclear cells. In addition to these there are many plasma cells, polymorphonuclear leukocytes, eosinophiles and a few giant cells.

The lymph nodes have a lesion histologically similar to that of the tracheal ulcer. In addition the structure of many of the nodes is completely replaced by fibrous tissue. There is also some deposition of carbon particles present.

The tumor of the liver has the microscopic structure of a granuloma. There is a central area of necrosis and a marginal zone of in-

flammatory reaction. At the periphery of the lesion the fibrous tissue is greatly increased and is compressing the liver cells, which are atrophied. Here and there in the tissue the bile ducts have proliferated. At the very margin of the necrotic area there are many newly formed capillaries which are filled with blood. About these and in the tissue adjacent to the necrosis are numerous cells of inflammatory origin. These are fibroblasts, epithelioid cells, lymphocytes, mononuclear cells, polymorphonuclear leukocytes and a few plasma cells. Giant cells are present but few in number. Sections from all of these tissues were stained for tubercle bacilli but none was found. Preparations were made to demonstrate *Treponema pallidum*, for which a modification of Warthin's technique [5] was employed. None was demonstrated (Fig. 5).

The histological studies of the lesions of the other organs are not of sufficiently unusual character or importance to detail their description here. The following gross and microscopic anatomical diagnoses were made.

General Diagnoses: Tertiary syphilis; gumma of the trachea; tracheo-esophageal fistula (syphilitic); chronic syphilitic lymphadenitis; gumma of the mediastinal lymph nodes; early aneurysm of the aorta; bronchopneumonia; acute suppurative bronchitis; myocardial fibrosis; gumma of the liver; passive congestion of the liver, spleen and kidneys; subserous fibromyoma of the uterus, and chronic salpingo-oöphoritis (non-tuberculous).

Discussion

The syphilitic nature of the lesion is established, we believe, beyond reasonable doubt. The strongly positive Wassermann and Kahn tests, the concomitant gumma of the liver, incipient aortic aneurysm, gummas of the lymph nodes, absence of tuberculous lesions in the lung or elsewhere, and the failure to find tubercle bacilli in repeated sputum examinations or in the sections of the tissue lesion, make up the evidence upon which the diagnosis rests. In addition, the histological character of the lesions is of great importance. The rather marked increase of fibrous tissue, the vascularity of the lesions, scarcity of giant cells and general lack of orderliness in the marginal cellular area, differentiate it fairly well from the tubercle. Like MacCallum [6] we are not convinced that a few giant

cells in these gummatous lesions mean an associated tuberculous lesion, as Baumgarten [7] believes.

The combination of tracheo-esophageal fistula, gummas of the liver and lymph nodes, and an incipient aneurysm is quite extraordinary. As far as we have been able to determine from the literature this case is the only one with so many concomitant syphilitic lesions. Von Fraenkel [8] quotes a case reported by Curschman in which gumma of the liver was an associated lesion. Berger, as quoted by Conner,[3] reports an instance in which gumma of the testicle was also present. Up to the time of death the gumma of the trachea and the subsequent fistula were the only lesions that produced symptoms. The lesion of the liver and the aneurysm were discovered at autopsy.

Summary

A case of tracheo-esophageal fistula of syphilitic origin is reported. Other lesions present were gummas of the liver and lymph nodes and incipient aneurysm of the aorta.

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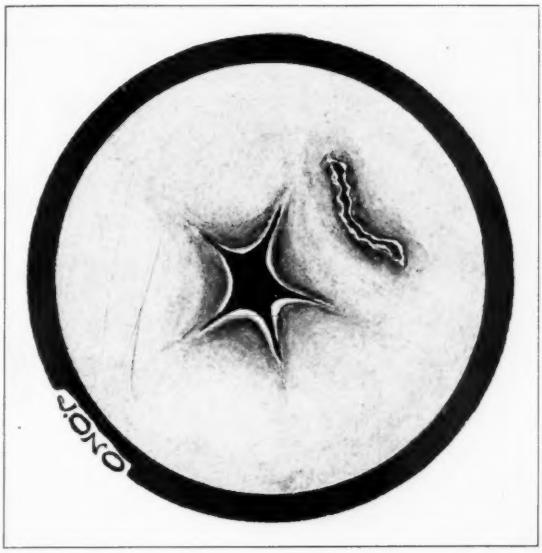
DESCRIPTION OF PLATES

Plate 101

Fig. 1. Esophagoscopic appearance of the fistula.

Fig. 2. Syphilitic ulceration of the trachea.



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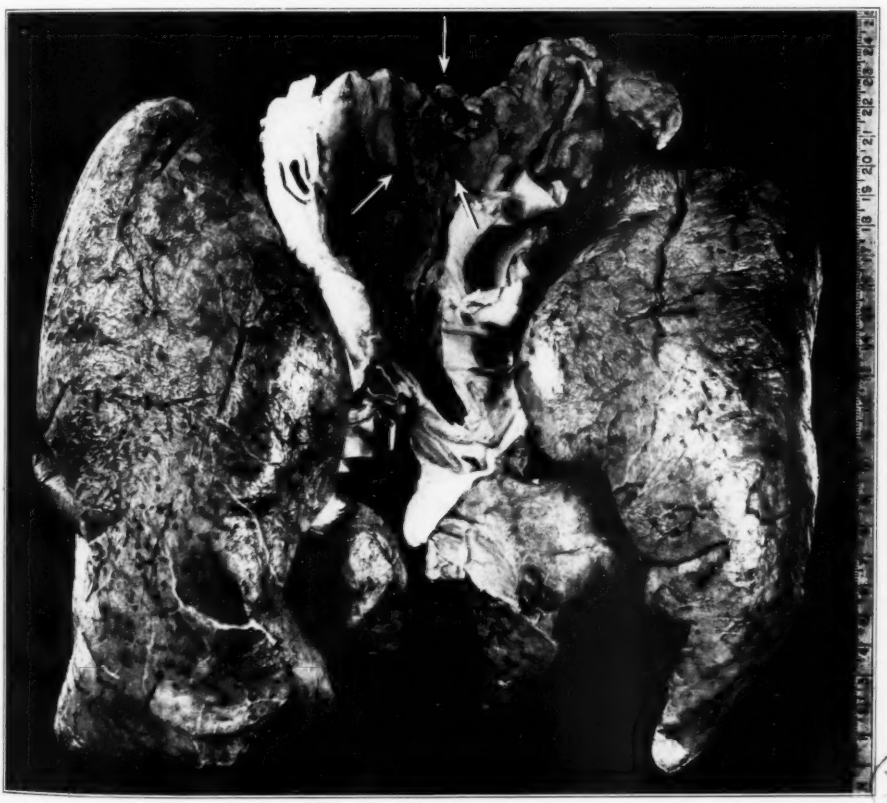


2

Bucher and Ono Tracheo-Esophageal Fistula of Syphilitic Origin

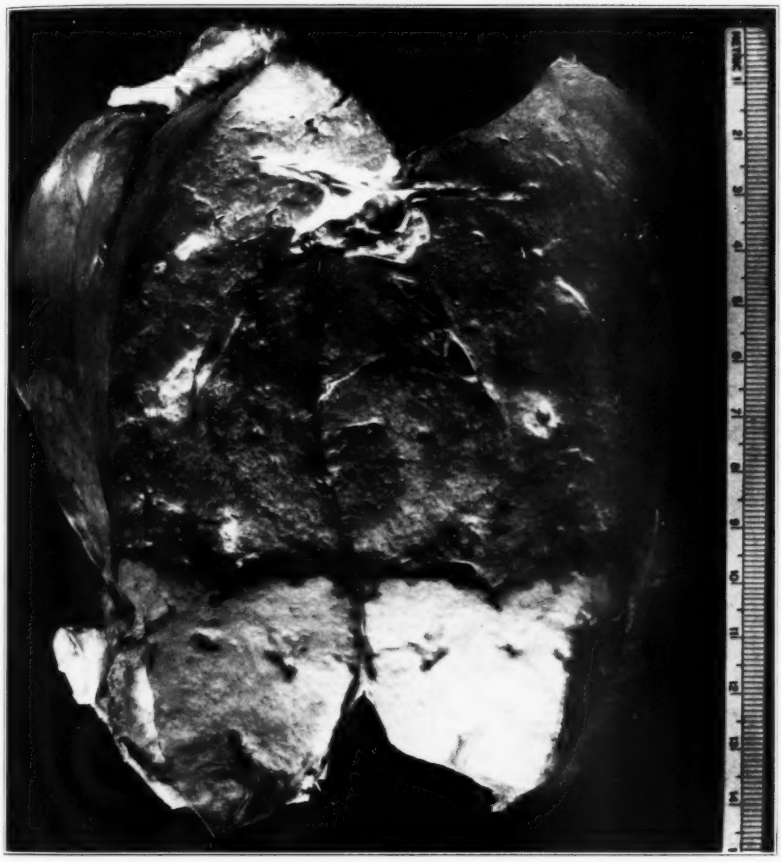
PLATE 102

FIG. 3. Stoma of the tracheo-esophageal fistula in the esophagus.

PLATE 103

FIG. 4. Gumma of the liver.

FIG. 5. (*a*) Microscopic section of the gumma of the liver. (*b*) Microscopic section of the tracheal ulcer. (*c*) Microscopic section of the thoracic lymph node.



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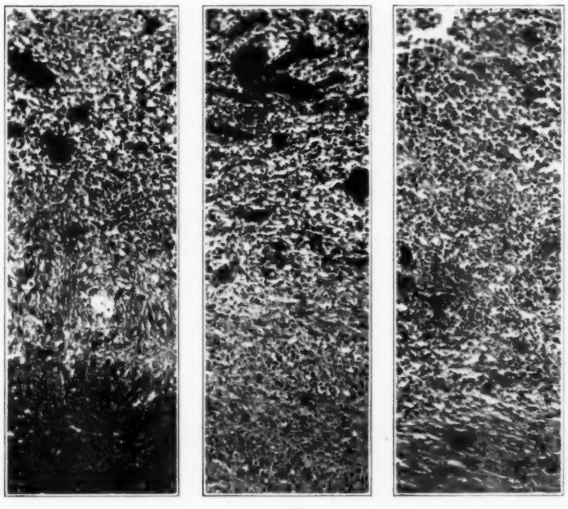

5a 5b 5c

Bucher and Ono Tracheo-Esophageal Fistula of Syphilitic Origin

MYXOMA OF THE HEART VALVES *

REPORT OF A CASE

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Although all tumors of the heart are rare, primary tumors of the heart do occur in the form of either sarcoma, fibroma, lipoma, myoma or myxoma, or combinations of two or more of these. With the exception of sarcoma these tumors tend to be benign. There has been considerable confusion and controversy about the classification of myxoma and fibroma of the heart ever since Czapek [1] asserted that many of the alleged myxomas of the heart were really organized thrombi. Thorel,[2] Stahr,[3] and Husten [4] concurred with Czapek in this opinion, while Ribbert,[5] Link,[6] Mandelstamm,[7] and Hagedorn [8] presented strong arguments in favor of the neoplastic nature of these tumors. The differentiation is made difficult by the microscopic similarity between myxoma and organized thrombi. Both show a homogeneous, poorly cellular matrix, and both are covered with endothelium. They differ, however, in that the myxomas are avascular, and usually have mucin and elastic fibers present, while organized thrombi are vascular, generally contain degenerated blood pigment, and have neither mucin nor elastic fibers. When these differences are considered, along with the gross appearance, there is little room for argument, for the organized thrombus is typically a smooth flat tumor with a broad base and more or less evidence of contracture and scarring, while the myxoma is a pedunculated, lobulated tumor with a narrow base and no evidence of contracture.

Although myxomas have been found in all chambers of the heart the sites of predilection are the interauricular septum of the left auricle and the heart valves. The myxomas that are found on the heart wall and septum are characteristically large tumors that may grow to a size sufficient to fill the entire chamber in which they are located and cause valvular insufficiency by occlusion of the valves.

* Received for publication November 15, 1933.

Choisy [9] first described a heart polyp of the left auricle situated in the valvula foraminis ovalis. Within the next few years similar cases were reported by de Puisaye,[10] LeGendre,[11] and Proust.[12] These tumors had the lobulated appearance and microscopic peculiarities of the myxoma. The valvular myxomas are more uncommon than those in the heart wall and are much smaller, varying from 6 to 15 mm. in diameter. They resemble the larger growths in that they are pedunculated, but show a characteristic papillary structure which the larger ones do not have. They are found on all the heart valves but are slightly more common on the tricuspid valve.

A total of 22 myxomas located on the heart valves have been reported. These are tabulated in the accompanying table. A consideration of the table will show that these tumors may occur at any age from birth to old age, and in either sex. As a rule, they are found accidentally at autopsy, having failed to give rise to clinical signs or symptoms during life.

Report of Case

Clinical History: A. H., a negress, 62 years of age, was admitted to St. Luke's Hospital on Dec. 26, 1932 because of failing vision in both eyes. Except for a pelvic operation 8 years before she had always been well.

The patient was an obese colored female with normal vital signs and, except for the eyes, normal physical findings. The pupils were equal, but both lenses showed large opacities. After a preliminary iridectomy the patient was discharged and readmitted 3 weeks later for a right cataract extraction, which was uneventful. On the 6th postoperative day she suddenly collapsed and showed the typical picture of circulatory failure, the blood pressure being 60/40 and the pulse feeble. Roentgenograms and electrocardiograms offered no assistance in making a diagnosis. She became steadily worse and died the next day.

Postmortem Examination

The body was that of an obese colored female. The positive findings were limited to the heart and pericardial sac. On opening the pericardial sac about 100 cc. of cloudy fluid were seen. The pericardium was hemorrhagic, and covered with small tags of fibrin. The heart weighed 325 gm. and the left ventricle measured 1.5 cm. in thickness. None of the valves was thickened, and there was no evidence of endocarditis. On the anterior cusp of the tricuspid valve, situated in the middle of the valve and about 5 mm. from its

TABLE I

Summary of Previously Reported Valvular Myxomas

Author	Year	Type of Tumor	Situation	Age	Sex	Remarks
				yrs.		
Luschka[13]	1857	Myxoma?	Pulmonic	40	F	
Curtis[14]	1871	Myxoma	Mitral	83	F	Old endocarditis present. Author considered it inflammatory
Debove[15]	1873	Myxoma	Tricuspid			
Ribbert[5]	1897	Myxoma	Pulmonic	38	M	All were highly papillary
			Tricuspid	"	"	
			"	"	"	
			"	"	"	
Guth[16]	1898	Papillary myxoma	Tricuspid	54	F	Other valves normal
Simmonds[17]	1901	Papillary fibroma	Tricuspid	25	..	Endocardium thickened. Multiple fibromas in walls
Reitmann[18]	1905	Hyalofibroma	Pulmonic	74	M	Papillary; matrix very poor in cells
Leonhardt[19]	1905	Myxoma	Mitral	26	F	Valve thickened
Djewitsky[20]	1906	Myxoma	Aortic	38	M	
Blumgart[21]	1907	Fibromyxoma	Mitral	86	F	
Hagedorn[8]	1908	Myxoma	Mitral	Middle aged	M	Patient had a hypernephroma
Koechlin[22]	1908	Myxoma	Pulmonic	19	F	Koechlin considered these tumors to be Lambl excrescences
			"	53	"	
			Aortic	60	"	
Forel[23]	1910	Fibroma	Aortic			
Dean and Falconer[24]	1913	Myxoma	Pulmonic	53	M	Some thickening of cusp
Kornfeld[25]	1928	Myxoma	Pulmonic	68	M	
Abrahamer[26]	1931	Myxoma	Pulmonic	Newborn infant		Similar growth on tricuspid
			Aortic	" "		

edge, was a small spherical tumor measuring 6 mm. in diameter and projecting 4 mm. above the surface of the valve, to which it was attached by a short wide pedicle. Its surface was finely nodular and it had a translucent gelatinous appearance (Fig. 1). The coronaries were thin and showed no evidence of sclerosis.

Permission for examination of the brain was not obtained, but it was assumed that the patient died of a cerebral accident.

Microscopic Examination

The cusp containing the tumor was excised, and sections were prepared with hematoxylin and eosin, Van Gieson's, thionin, mucicarmine, and Weigert's elastic tissue stains.

There is no microscopic thickening of the cusp, and there is well marked differentiation of the layers of the valve. A narrow pedicle attaches the tumor to the cusp, and the endothelium and elastic laminae are reflected onto the pedicle. The pedicle very shortly spreads out, and divides into two main limbs, from each of which numerous papilliform processes arise (Fig. 2).

Stained with hematoxylin and eosin the tumor matrix is pink. There is a complete endothelial covering over the entire tumor. The papillae contain only a few cells and there are no blood vessels present; the pedicle, on the other hand, contains many stellate and spindle-shaped cells which have fine processes extending from them into the fine fibrillar groundwork present throughout the entire tumor (Fig. 3).

The test for mucin with thionin is only faintly positive; the stain with mucicarmine, however, is strongly positive, the entire matrix taking a deep red stain. Van Gieson's connective tissue stain shows a large number of coarse, pink-staining fibrils in the pedicle which extend into the papillae and become lost. The presence of elastic fibers is proved by Weigert's elastic tissue stain, which shows a large number of vertically placed, blue-staining fibers throughout the entire tumor.

The tumor presented then is one arising by a well circumscribed pedicle from the tricuspid valve, and which is free of any inflammatory change. The tumor contains mucin and elastic fibers. Part of this tumor, however, consists of hyaline connective tissue.

Discussion and Summary

When a tumor is found on a heart valve it may be either a Lambl excrescence, a product of an inflammatory reaction or a neoplasm. Organized thrombi do not have to be considered, since they do not occur on valves.

Lambl [27] first described the excrescences which bear his name as thread-like structures 2 to 3 mm. in diameter, occuring on the aortic valve. These are papillary in form and resemble myxomas microscopically. Koechlin [22] felt that all the valvular myxomas should be called Lambl excrescences. In examining 150 bodies he found thread-like growths on the valves in 20 per cent of the cases. However, all the Lambl excrescences reported have occurred on the aortic cusps with the exception of 2 cases reported by Koechlin,[22] in which they were found on the mitral valves, and in these there was evidence of an old endocarditis present. Whereas Lambl excrescences are generally found in a heart exhibiting endocardial changes myxomas occur in hearts with a normal endocardium. Moreover, myxomas are larger, measuring 6 to 10 mm. in diameter, and are more compact. Inflammatory growths on the valves are generally accompanied by gross thickening of the valves, destruction of valvular contours, and by microscopic scarring.

If it be assumed, as did Ribbert [5] and Leonhardt,[19] that true mucin occurs in a true tumor, and could come about in no other way, then these valvular growths can be readily differentiated by the various chemical and color reactions characteristic of mucin. Ribbert believed that the myxomas were true tumors which originated from embryonic connective tissue, and that ultimately the mucoid tissue gave rise to fibrous tissue. The fibroma described by Hagedorn [8] represents the endpoint in this development.

Reitmann,[18] on the contrary, believing that the myxomas represented a stage of degeneration of connective tissue tumors, designated his case as a hyalofibroma.

Whether the myxomas are really neoplasms, or whether they represent a degenerative process in connective tissue growths is still open to question. The fact is, however, that they do represent a group of tumors that occur in the heart, and have rather uniform morphological characteristics.

NOTE. I am greatly indebted to Dr. Francis Carter Wood and to Dr. Leila C. Knox for their encouragement and criticism throughout this work.

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DESCRIPTION OF PLATES

PLATE 104

FIG. 1. Heart showing a myxoma (A) attached to the tricuspid valve on the auricular surface. The right ventricle has been cut open. Approximately natural size.

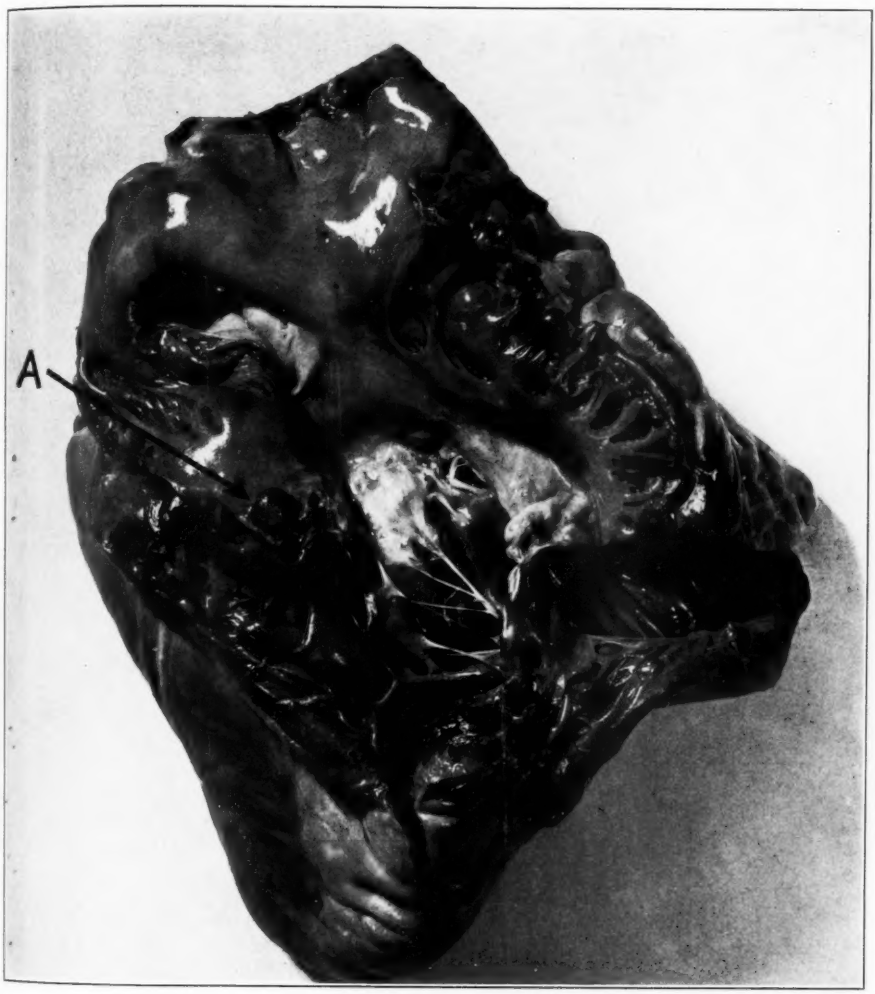

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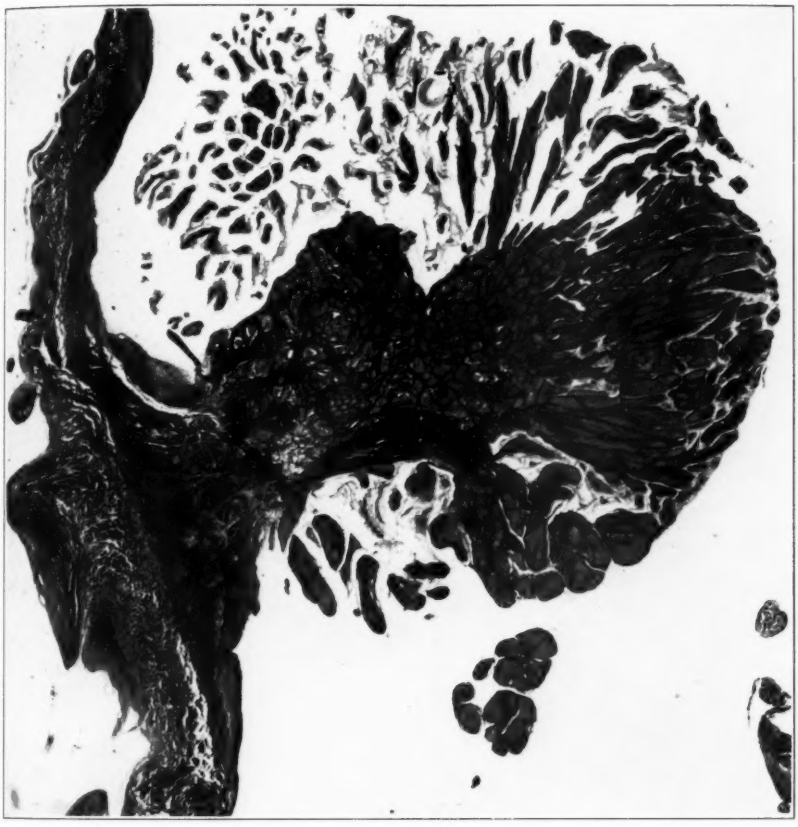
PLATE 105

FIG. 2. Microscopic section showing a myxoma attached to the tricuspid valve. × 18.

FIG. 3. Cross-section of several papillae of the myxoma showing the endothelial cells covering them, and the poorly cellular matrix. × 100.



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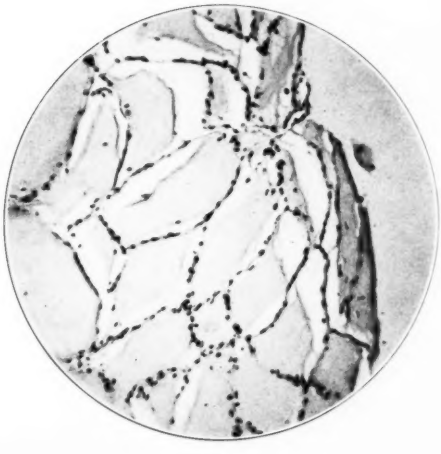

3

MICROSCOPIC METASTASES IN THE THYROID GLAND *

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Metastases from malignant growths to the thyroid gland, although somewhat infrequent, probably occur more often than has been supposed. In a series of 521 autopsies on patients dying from carcinoma Müller [1] found gross metastases in the thyroid gland in 1.5 per cent. In another group of 102 postmortem examinations on patients dying from sarcoma he found metastases in 3.1 per cent of the thyroid glands. Kitain [2] found metastases in 3.1 per cent of the thyroid glands from autopsies on individuals dying of carcinoma. In 170 consecutive autopsies on persons with malignant tumors Willis [3] found thyroid metastases in 5.2 per cent. He mentions that only by careful routine sectioning of the gland was it possible for him to find such a high incidence of metastases. In half of his 10 cases a casual bilateral section of the gland would have failed to disclose these metastatic areas. Wegelin [4] does not consider metastasis to the thyroid gland an exceptional rarity, for he found it frequently in the material examined at the Pathological Institute of the University of Bern.

It is a well known fact that certain types of malignant conditions select certain regions for their metastatic growths. Carcinoma of the thyroid, kidney and prostate seems to have a selective affinity for metastasizing to bone, whereas carcinoma of the breast has a tendency to metastasize to the lungs.

The causative factor for this peculiar type of selective metastasis is not definitely established. Various mechanical theories have been suggested to account for the lodgement of malignant cells in certain areas. Likewise, to chemical factors has been attributed the principle rôle in determining the sites for the development of metastatic growths. Paget [5] speaks of certain tissues as being "congenial soil," whereas Ewing [6] has expressed an opinion opposed to this when he mentions that no organ is more adapted than any other for the growth of embolic tumor cells.

* Received for publication December 1, 1933.

Among the 57 autopsies with secondary carcinomatous growths in the thyroid gland reviewed by Willis, the primary tumor was most frequent in the breast. Next in frequency were the malignant melanomas, and third, carcinoma of the lungs and bronchi. He suggested that the high incidence of this phenomenon secondary to carcinoma of the breast was in all probability due to the greater frequency of that disease. On this assumption it should be possible to give first and second place to malignant melanoma and carcinoma of the lungs, both of which are relatively infrequent types of malignancy in the thyroid. Kaufmann[7] and Eiselt[8] found a relatively high incidence in malignant melanoma. In 34 autopsies with thyroid metastases Wegelin found 6 arising from carcinoma of the esophagus, 5 from carcinoma of the lung, 5 from melanosarcoma, 4 from carcinoma of the stomach, and the others from a variety of sources. He mentions that in the majority of instances the metastatic growths in the thyroid are visible as definitely circumscribed nodules.

Of all the men who have reported cases of malignant disease with metastases to the thyroid, none of them has found metastases that were not grossly visible. This paper is written for the purpose of demonstrating that microscopic metastases may occur in the thyroid gland without any macroscopic evidence of their presence.

Among 89 postmortem examinations from the department of pathology of the University of Minnesota on patients dying from malignant tumors there were embolic tumor cells within the thyroid gland in 9. The thyroid glands from all these patients were examined grossly and microscopically. Five of these showed gross metastases: 2 were from patients dying of malignant melanoma, 1 from carcinoma of the lung, 1 from lymphosarcoma, and 1 from carcinoma of the breast. These carcinomatous nodules were easily identified on macroscopic inspection of the gland. The remaining 4 cases presented, on gross inspection, no evidence of any tumor tissue or anything else to suggest the presence of malignant invasion. The essential details in the case histories of these cases are reported in the following case reports.

Case Reports

Case 1. Mrs. E. F., (A–29–595), aged 44 years, was admitted to the Minnesota General Hospital on March 28, 1929. A radical operation for carcinoma of the breast had been performed 3 months previously. The patient died on April 17, 1929.

The postmortem examination revealed numerous metastatic nodules in the bones, liver and lungs. The thyroid gland weighed 34 gm., and appeared normal in structure, with the exception of a few small colloid nodules and one large parenchymatous nodule measuring 15 mm. in diameter. There was no area, on gross inspection, that suggested malignant tissue.

Microscopic examination of the thyroid tissue reveals normal acini. Scattered throughout in the interlobular septa there are groups of large, irregularly shaped, deeply stained, closely packed cells, among which occasional mitotic figures can be seen. The nuclei are large and the cells contain a small amount of cytoplasm (Figs. 1 and 2).

The histological structure of the metastatic nodules from the liver is similar to the carcinomatous tissue observed in the interlobular septa of the thyroid.

CASE 2. Mr. F. R., (A–29–1323), case history not available.

The thyroid gland weighed 27 gm. It was diffusely homogeneous in structure and appeared to be entirely normal. Neither nodules nor areas suggesting a malignant condition could be identified on gross inspection.

Microscopic examination reveals normal thyroid acini. Here and there in the interlobular septa can be seen groups of large, pale staining, round and polyhedral cells. Some of these have large vacuolated nuclei and a large amount of cytoplasm. Mitotic figures are moderately abundant (Figs. 3 and 4).

CASE 3. Mrs. J. E., (A–29–285), aged 67 years, was admitted to the Ancker Hospital on Feb. 14, 1929, complaining of difficulty in breathing, dyspnea and a productive cough of 5 days duration. The most pronounced symptom was the cough. Dyspnea became more severe 2 days prior to admission to the hospital. She also gave a history of having had an ulcerated lesion on the right malar region for the past 9 months.

On physical examination a superficial, ulcerated lesion 1 cm. in diameter was found over the right malar region and a similar lesion over the right mandible. Examination of the chest revealed coarse moist râles over both lungs, but no evidence of consolidation. The blood pressure was 230/120. A clinical diagnosis of acute bronchitis, laryngitis, hypertension and carcinoma of the cheek was made.

The X-ray report of the chest disclosed infiltration and fibrosis in both lungs. This was suggestive of pneumoconiosis, associated with tuberculosis, and possibly bronchiectasis in both apices.

The patient became rapidly weaker and died on Feb. 16, 1929.

The postmortem examination disclosed the indurated, ulcerated lesion on the cheek, but no section was made of this to determine its exact nature. The liver was enlarged. Some old adhesions were found in the pleural cavity. There were 400 cc. of fluid in the left pleural cavity and 200 cc. of blood-tinged fluid in the right pleural cavity. A large focus of consolidation was present in the right lung, and several large, anthracotic and caseous lymph nodes were present at the hilum of both lungs. Microscopic examination of these tissues shows carcinoma. The lung was considered to be the site of the primary lesion. Its gross appearance did not suggest carcinoma and its presence was not suspected until sections were examined. The thyroid gland weighed 22 gm. Its structure was uniform. There were no nodules or any areas that could be interpreted as malignant on gross inspection.

The microscopic structure of the thyroid acini appears to be normal. Scattered throughout, in the interlobular septa, are found groups of large clear cells with enlarged granular nuclei and a pale staining cytoplasm. Mitotic figures are occasionally seen. Intercellular bridges are not observed. The cells are similar to those found in the sections from the lungs (Fig. 5).

CASE 4. Mrs. L. K., (A-30-754), aged 47 years, was admitted to the Ancker Hospital on April 25, 1930, complaining of distention with gas, loss of appetite and exhaustion for the past 6 weeks. She had had no pain, nausea, vomiting, or food distress.

Physical examination disclosed a slight bilateral exophthalmos. The liver was found to extend 7 cm. below the costal margin. A systolic murmur was heard along the sternum in the second left interspace. The knee jerks were hyperactive; Babinski test, normal.

On May 1, 1930, palpation revealed fullness in the left upper quadrant of the abdomen. The patient was drowsy and very restless. On May 4th she became stuporous and could not be aroused. She developed a left sided hemiplegia and became incontinent. The knee reflexes became increased on the left. The Babinski test was positive on the left. On May 8th there was definite neck rigidity. Pure blood was found in the spinal fluid. Two hard lumps appeared on the head, one of which was located over the left frontal bone, the other in the right parietal region. On May 12th many fine râles were heard in the lungs. The temperature varied from normal to 102.4 F. The pulse ranged from 80 to 140. The hemoglobin was 55 per cent, erythrocytes 3,290,000, lymphocytes 5600, differential count normal.

The patient became progressively worse and died on May 12, 1930.

At the postmortem examination it was found that the two nodules on the head were metastases in the cranium, extending into the

cranial cavity. Numerous grayish white nodules, which proved to be metastatic tumor tissue, were found within the substance of the liver, occupying approximately one-fifth of the liver volume. The gall-bladder contained numerous faceted stones and its wall was definitely thickened and almost cartilaginous. This tissue proved to be carcinomatous and was thought to be the primary lesion. There was an area of pneumonic consolidation in the lower lobe of the left lung. Numerous metastatic nodules were found around the otherwise normal pancreas. The preaortic and mesenteric lymph nodes were invaded with metastatic tumor tissue. Pus was found beneath the arachnoid over the right hemisphere and there was softening of the entire right half of the brain. The thyroid gland was homogeneous in structure and appeared grossly to be entirely normal. There was nothing to indicate the presence of metastatic tumor tissue.

Microscopically the thyroid acini appear normal. Scattered throughout in the interlobular septa can be seen groups of large basophilic cells with large, round and polyhedral nuclei, and a moderate amount of pale staining cytoplasm. In other places these cells are darker and more spindle-shaped. Mitotic figures are abundant. The malignant cells are similar to those found in the gall-bladder, liver and lymph nodes (Fig. 6).

Discussion and Conclusions

These cases illustrate the occurrence of a type of metastasis that is often thought of as a precursor to the gross metastatic nodules, but which is infrequently seen in pathological specimens, probably because of the prevailing tendency to make histological sections only where gross pathological changes are visible.

In all these cases macroscopic inspection of the gross specimen failed to give any intimation that there were metastases in the thyroid gland. Although it is probable that this type of metastasis occurs frequently, a search of the literature has failed to reveal any reports of a similar nature.

The incidence of gross metastases in the thyroid gland, as reported by other writers, ranges from 1.5 per cent to 5 per cent. These figures compare favorably with those in this report when the cases with microscopic metastases are not included, but otherwise

the incidence approaches 10 per cent. It may be assumed then that microscopic metastases in the thyroid gland occur almost as frequently as gross metastases in this organ, and in all probability the incidence of metastasis is much greater than has been reported in the literature.

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DESCRIPTION OF PLATE

PLATE 106

FIG. 1. Case 1. Photomicrograph showing the microscopic structure of the thyroid gland with groups of deeply stained carcinomatous cells invading the interlobular septa.

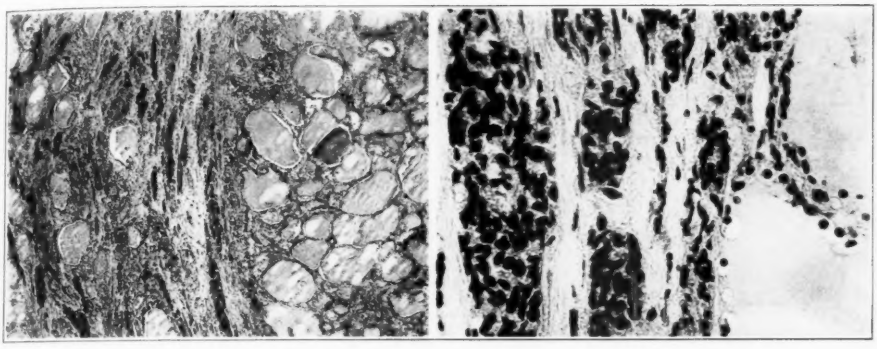
FIG. 2. Case 1. High power magnification from the same section as Fig. 1, depicting a few of the groups of tumor cells. Two thyroid acini are seen at the right of the picture.

FIG. 3. Case 2. Photomicrograph showing interlobular septa filled with large and small groups of tumor cells. Thyroid acini normal.

FIG. 4. Case 2. High power magnification from the same section as Fig. 3. Mitotic figures are seen on the left side.

FIG. 5. Case 3. Photomicrograph showing thyroid acini which appear normal. The interlobular septa is occupied by groups of carcinomatous cells.

FIG. 6. Case 4. Photomicrograph showing interlobular septa filled with densely packed groups of tumor cells. Thyroid acini appear normal.



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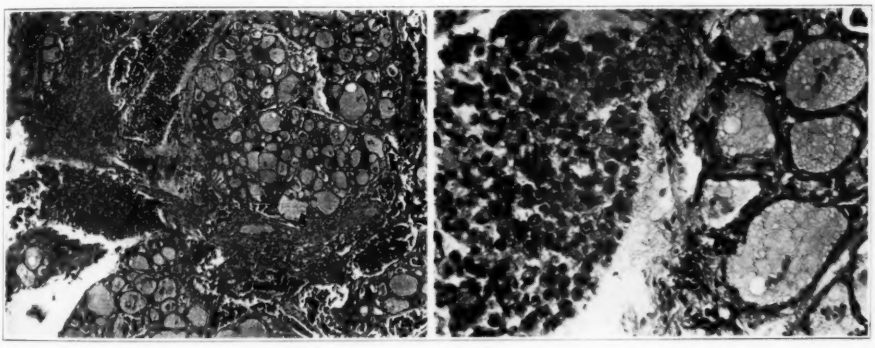

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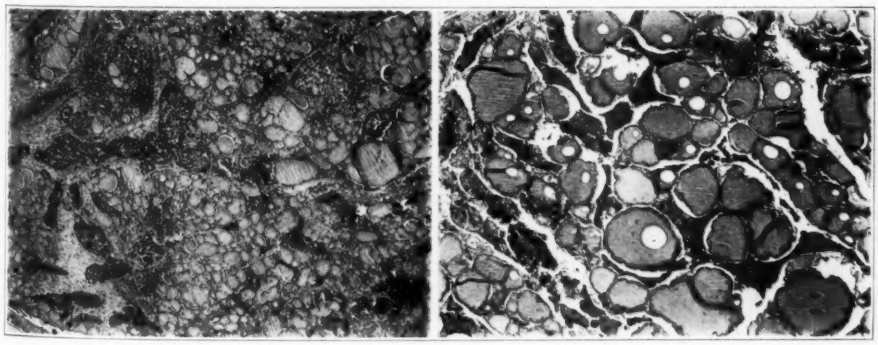

5 6

SARCOSPORIDIA IN THE MYOCARDIUM OF A PREMATURE INFANT *

REPORT OF A CASE

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REVIEW OF LITERATURE

Scott,[1] in a critical review of the sarcosporidia, summarizes our present knowledge of this group of parasites. The sarcosporidia are protozoa of unclassified position, found almost exclusively in the striated muscles of birds, reptiles and mammals, including man. Miescher [2] in 1843 first described the parasite in the muscles of the mouse. The genus *Sarcocystis* was created in 1882 by Lankester.[3] Since then at least 43 species have been described from an almost equal number of hosts. Whether these are all true species or whether only one or a limited number of species exists is not known. Probably there are several species, since some are transmitted to other hosts with difficulty. Darling [4] and others, however, have shown that at least two hosts may be parasitized by the same species, although in the transfer to a new host certain morphological changes occur in the parasite.

In the opinion of most investigators natural infection is through the digestive tract by means of contaminated food, although the exact details are not known. The first successful transmission by feeding was obtained by Theobald Smith [5] in 1901, who was able to infect 63.6 per cent of gray and white mice by feeding them muscle from previously infected animals (mice). Nègre [6] and Crawley [7] have both shown that feces of infected mice are capable of causing the disease when ingested by other mice. The infective stage in this instance is a spore, resistant to heat and drying. Erdmann [8] stated that in the intestinal tract the spore liberates a small ameboid form which penetrates the epithelium. It then multiplies within the various portions of the gut wall and surrounding lymphatics to ap-

* Received for publication December 6, 1933.

pear finally 28 to 30 days later in the striated muscles. The various stages within the striated muscle fibers are known largely from the works of Theobald Smith,[5] Scott [1] and others.

Human Cases

The first probable instance of infection with sarcosporidia in man was reported by Lindemann [9] in 1868, as occurring in the heart valves and myocardium. These were interpreted as gregarines but Fantham, Stevens and Theobald [10] consider them sarcosporidia. The latter authors doubt the authenticity of Rosenberg's case of sarcosporidia,[11] occurring in the mitral papillary muscles in the heart of a woman 40 years of age, dying with pleuritis and endocarditis. Fantham, Stevens and Theobald also cite 2 cases reported by Koch in 1887, and by Vuillemin in 1894, respectively. The location of the parasite in the case reported by Koch was not stated, while in the case reported by Vuillemin the parasite occurred in the voluntary muscles of a patient dying of tuberculosis. Cone's report [12] in 1922 of the parasites associated with yeasts in multiple bone cysts is a disputed case. The undoubted cases occurring in the human are best summarized in the accompanying table.

It is the purpose of this paper to report the incidental finding of a sarcosporidial infection in the myocardium of a premature infant.

Report of Case

Clinical History: A 10 day old, white, female infant, which was born 2 months prematurely, was brought to the hospital with a history of having had loose stools since birth and ulcerations of the buttocks for 2 days. Since birth it had been fed by a medicine dropper on a very weak, whole milk, dextrimaltose and water formula. Physical examination revealed emaciation, and ulceration of the buttocks. On the 6th day after admission an omphalitis became apparent, with discharge of thick yellow purulent material from the umbilicus. The latter, along with the diarrhea, became progressively worse and the baby died 16 days after admission with signs of a terminal bronchopneumonia and cardiac dilatation.

Postmortem Examination

Postmortem examination (A–32–163) revealed a poorly nourished premature infant measuring 40 cm. in length. Purulent exudate filled the proximal end of the umbilical vein. Multiple abscesses yielding *Staphylococcus aureus* and *Bacillus coli* occurred in the cortex

of both cerebral hemispheres, and to a less extent throughout the brain. The lungs showed a bilateral terminal bronchopneumonia with atelectasis and compensatory emphysema. Microscopic foci of necrosis were found in the myocardium, liver and spinal cord. Small

TABLE I

Undoubted Cases of Sarcosporidiosis *

Author	Year	Age	Organ
		yrs.	
Kartulis [13]	1893	Adult	Abdominal muscles
Baraban and Saint-Remy [14]	1894	Adult	Laryngeal muscles
Darling [15]	1909	20	Biceps
Darling [16]	1920	30	Tongue
Manifold [17]	1924	Adult	Myocardium
Lambert [18]	1927	32	Myocardium
** Vasudevan [19]	1927	Adult	Pectoral muscles
** Naidu [20]	1928	55	Pectoral muscles
Bonne and Soewandi [21]	1929	Adult	Cavernous hemangioma
Hewitt [22]	1933	Adult	Myocardium

* An additional human case of sarcosporidiosis, reported by Price, R. M., in the *J. Kansas M. Soc.*, **34**, 132–135, has come to my attention since this paper was written.
** These two possibly represent reports of the same case.

TABLE II

Comparative Measurements of Parasite in μ made by Various Authors

Author	Cysts		Spores	
	Length	Diameter	Length	Diameter
Baraban and Saint-Remy [14]	150–1600	77–168	...	...
Darling (Case 1) [15]	84	27	4.0	1.0
Manifold [17]	37–57	26–45	10.9	1.6
Lambert [18]	82	31	7.2	2–2.5
Vasudevan [19]	5.3 cm.	322	3.3	1.6
Naidu [20]	195–240	...	12.7	4.4
Hewitt [22]	190	50	5.0	2.0
Hertig	11.2–45	7.4–13	3.7	1.8

foci of purulent meningitis were found over the cerebral cortex, as well as a slight acute inflammatory reaction throughout the brain substance. The lungs, in addition to bronchopneumonia, gave microscopic evidence of aspirated amniotic sac contents. The pancreas showed slight, diffuse, acute inflammation with inspissation

of secretion in the finer ducts. Moderate numbers of sarcosporidia, including many in the early stages, were found microscopically in the myocardium. In no instance was there cellular infiltration in response to the presence of the parasite, although independent foci of necrosis were found. Examination of other striated muscle (diaphragm) revealed no parasites.

Description of Parasite

The lateral portion of the left ventricle, taken during a routine postmortem examination, was the source of the material. The parasites were identified by Professor S. B. Wolbach in studying the microscopic preparations. Sections stained by eosin and methylene blue, from material fixed in Zenker's fluid, gave the clearest histological detail, although hematoxylin and eosin, Giemsa, iron hematoxylin and Foot's reticulum stains were also made. The parasites were moderately numerous throughout the myocardium, averaging 1 per low power field. They lay within the myocardial fibers as sharply demarcated, oval bodies varying from 7.4 by 11.2μ to 13 by 45μ, with the long axis parallel to that of the surrounding fiber. The parasites, as seen in individual sections, were composed of closely packed, oval or spindle-shaped spores varying from 6 to 100 in number. No definite wall could be seen in the forms possessing less than 13 spores, although beyond that stage a hyaline wall or capsule averaging less than 1μ in thickness was present. Occasionally, due to artifact, the cyst wall was pulled away from the surrounding muscle fiber and thus could readily be seen. No septa were seen in any of the forms studied. Slightly over 30 per cent of the parasites in this preparation contained from 6 to 13 spores, with the higher numbers predominating slightly, although approximately 10 per cent contained between 50 and 100 spores. The spores averaged 1.8 by 3.7μ in size. The basophilic nuclear mass was irregular and occupied an eccentric position, often filling one end of the spore. No nuclear membrane could be made out, although the spore membrane was quite definite. Very rarely a suggestion of a minute extranuclear basophilic mass could be seen at the end of the spore opposite the nucleus. At no place in the myocardium was there any inflammatory response to the parasites. The remainder of the myocardium was essentially negative, except for foci of necrosis associated with the staphylococcus septicemia.

Discussion

This case is of interest because of its occurrence in a premature infant and the early stage of development of the sarcosporidial cysts. Even in experimental studies it is uncommon to find sarcocysts with fewer than 8 spores, although moderate numbers of the forms studied here were of this type. The method of infection is unknown. However, since the infant was 26 days of age at death the infection could have been contracted shortly after birth, because the stage of development coincides very well with that seen in animals 26 to 29 days after ingestion of the infective spores. Theobald Smith [23] and Scott [1] state that intra-uterine infections do not occur in the lower animals, although this method cannot be ruled out in this case. Since mouse feces are known to be infective and since indigenous mouse infections may be common, this might have been a source of infection.

Summary

A case of sarcosporidiosis involving the myocardium of a 26 day old premature infant is reported. This was an incidental finding in the routine microscopic study of postmortem material. The mode of entrance of the parasites into the body cannot be stated with certainty.

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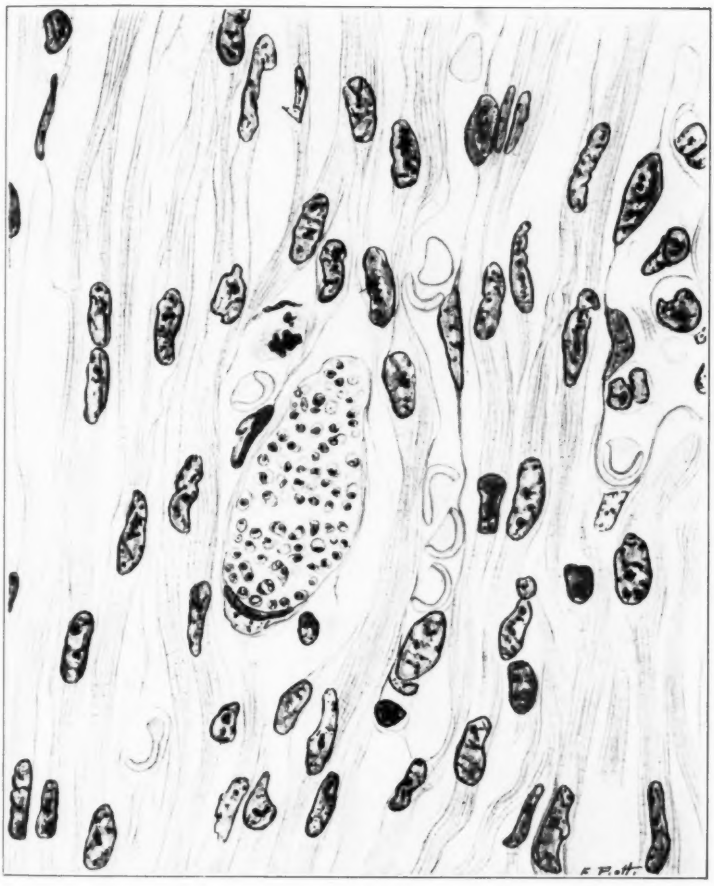
DESCRIPTION OF PLATE

PLATE 107

FIG. 1. Sarcosporidial cyst in the myocardium of a premature infant. × 1300.



1

OCCURRENCE OF AMYLOIDOSIS IN RABBITS EXPERIMENTALLY INFECTED WITH TUBERCULOSIS *

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Amyloid degeneration of the spleen, liver and kidneys has long been known to occur in the course of chronic wasting diseases, particularly in those in which suppuration is present. Chronic tuberculosis fulfills both of these conditions, and most cases of amyloid degeneration are found in this disease. Higuchi,[1] in assembling the autopsy findings of his own and of other European hospitals, found in a total of 72,050 autopsies 1169 cases of amyloid degeneration, of which 75.5 per cent were in tuberculous cases. Von Schrötter,[2] in a series of 3716 tuberculous autopsies, found 221, or approximately 6 per cent, affected with amyloid degeneration. In contrast to this are the figures published by Cummins [3] from a smaller series, in which he found 123 cases of amyloid degeneration in a total of 236 autopsies on tuberculous patients. It may be noted that Cummins stated in his publication that he used a specific stain (methyl violet) in examining the sections, whereas the above authors make no note of having used specific stains. Excellent reviews of the chemistry and of the histopathology, together with bibliographies of the older literature, have been published by Schmidt,[4] Wells and Long,[5] Edens [6,7] and Davidsohn.[8,9]

The material for the present study consisted of a group of 175 rabbits that had been infected with bovine tubercle bacilli and allowed to die of their infection. Gross and microscopic examination of the tissues was made in each case. Sections were stained with hematoxylin and eosin as routine, and in addition with azocarmine and aniline blue (Heidenhain's modification of Mallory's aniline blue stain) and, in a few instances, with Congo red according to the method of Bennhold.[10] The average length of life of the animals after infection was 6.7 months, with a lower limit of 2 weeks and an upper limit of 20 months (see Chart 1).

* Received for publication December 7, 1933.

Rabbits that died during the first 2 months after infection did not show any amyloid changes, despite the fact that they were suffering from a widespread tuberculosis. It may be noted in this connection that at this time caseation is not a pronounced factor. In the 3rd month the incidence of amyloidosis was 23 per cent, in the 4th month 55 per cent, and in the succeeding months between 60 per cent and 87 per cent. Of all the animals that survived beyond 2 months after infection 62 per cent showed amyloid degeneration of one or more organs, while of those that survived 8 months or

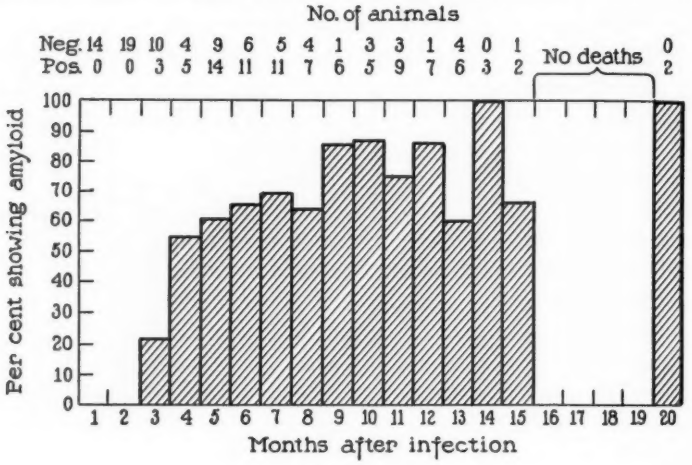

CHART 1

longer 75 per cent were involved. The incidence by months is graphically shown in Chart 1.

The spleen, liver, and kidneys showed the greatest involvement. The mesenteric lymph nodes were never extensively involved and in but a few cases was any amyloid found in the lymph nodes. The spleen was most frequently involved, and in a number of animals was the only organ affected. The early changes in the spleen consisted of a deposition of amyloid substance in the capillary walls at the edge or border of the malpighian corpuscles, forming in section a "rim" around the follicle. Later, extension occurred toward the center of the follicle, leaving finally the central artery with but a remnant of lymphoid tissue about it. The capillaries of the follicle, surrounded as they were by masses of amyloid substance, fre-

quently remained open and did not show constriction of their lumens. In approximately half of the cases the amyloid was not confined to the follicles but had also involved the pulp, so that the entire organ was a mass of amyloid material. There was no increase in weight of the spleens in the affected animals. The average spleen weight in the affected group was 1.5 gm., and in the unaffected group 1.56 gm.

In the liver amyloid deposits were found between the endothelium lining the liver sinusoids and the liver cells, and occasionally in the media of the central veins. In severe cases the liver cells were thinned out and practically obliterated by the encroachment of the amyloid substance upon them. No evidence of vascular obstruction was noted in any of the cases, although this feature, terminating in extensive hemorrhage and rupture of the liver, is common in "antitoxin" horses that develop amyloid disease of the liver.[11, 12]

The kidneys were found to be less frequently involved than the spleen or liver. Amyloid deposits were found in the capillary tufts of the glomeruli, sometimes involving but one loop and sometimes the entire glomerulus. In several severe cases all of the glomeruli were so involved, with attendant atrophy and degeneration of the tubules.

The kidneys and livers of these animals were not weighed at autopsy. There were no gross evidences of amyloidosis save for an increased firmness of the organ. Microscopically there was no evidence that in either the liver or kidney amyloid deposits tended to form in large aggregates.

The clinical course of the disease seemed not to be affected by the occurrence of amyloidosis. The changes in the white blood cells and the red blood cell count were not significantly different in the two groups. There was no increased cachexia in the affected group of animals. As regards the effect of amyloid degeneration of the spleen, liver and kidneys upon the length of survival of the animals after infection, it is seen in Chart 1 that the distribution of amyloidosis was not confined to any age group; and that the unaffected group (exclusive of those that died in the first 2 months) lived an average of 6.7 months, while the group in which amyloid was found survived an average of 8.5 months. These figures indicate that amyloid changes are a function of chronicity rather than an untoward incident or complication in the course of the infection. On the other hand, the examination of the autopsy records showed that

amyloid changes were more frequently found in those animals with extensive caseation. While the extent of pulmonary tuberculosis was approximately the same in the two groups, in the unaffected group only 23 per cent showed tuberculosis with caseation of the testicle and 64 per cent similar tuberculosis in the kidney. In the animals with amyloid changes, however, the testicles were involved in 96 per cent of the cases and the kidneys in 81 per cent. In rabbits experimentally infected with tuberculosis the testicles and kidneys are both prone to undergo rapid and extensive caseation and in severe cases may together contain 50 to 60 gm. of caseous material.

In but 3 cases did there appear to be sufficient involvement of the liver or kidney to account for the death of the animal. The fact that the group of animals that showed amyloid degeneration and more extensive lesions with caseation survived, as a group, longer (1.8 months) than the unaffected group is but an expression of the fact that amyloid degeneration is a function of chronicity, so that given indefinite survival the incidence would approach 100 per cent. The relation between extent of disease and survival time after infection with tuberculosis is by no means clear, and this is particularly true in experimental tuberculosis where the actual causes of death are frequently obscure.

Summary

1. Amyloid degeneration occurred in 52 per cent of 175 rabbits experimentally infected with bovine tubercle bacilli.

2. The occurrence of amyloidosis was restricted to animals surviving longer than 2 months after infection.

3. The frequency of occurrence was greatest after the 8th month (75 per cent).

4. The organs affected were the spleen, liver and kidney, the spleen being most frequently affected.

5. There was a uniform tendency for the deposition of amyloid to occur in those animals that showed the most extensive caseation of their lesions.

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A STUDY OF THE ACTION OF A FILTRABLE STAPHYLOCOCCAL TOXIN ON THE KIDNEYS OF NORMAL RABBITS *

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During a study of the action of a staphylococcal toxin on leukocytes *in vivo* and the subsequent changes produced in the femur bone marrow of normal rabbits [1] it was noted at the time of autopsy that some of the animals had extensive necroses of the cortical portion of the kidneys. It was thought that this observation deserved further study and this is the experiment to be submitted.

So far as we have been able to determine Neisser and Levaditi[2] in 1900 were the first to produce necrosis in the kidneys of rabbits by the intravenous injection of staphylococcal toxins. Following the publication of this work little attention was given to staphylococcal toxins until the revival of interest, which began a few years ago following the experiments of Julia T. Parker Weld. Subsequent to her reports we have been able to find only two references to necrosis in kidneys after the intravenous injections of staphylococcal toxins: one was by Weld and Gunther [3] in 1931, and the other was by Forssmann [4] in 1932. From a study of the literature it is evident that the kidney lesions produced by the intravenous injections of staphylococcal toxins have not received due consideration.

METHODS AND MATERIALS

Animals: The animals used in these experiments were normal adult rabbits.

Toxin: The toxin was prepared by the method described by Parker, Hopkins, and Gunther,[5] with a few unessential modifications.

Organism: A hemolytic *Staphylococcus aureus* was the organism from which the toxin was prepared. This staphylococcus had been isolated from the throat of a patient who presented the classical lesions found in agranulocytic angina. The cultures of this organism were grown on blood agar.

Procedure: The rabbits were given either a single intravenous injection of toxin each day until death, or the injections were made daily over a period of 10 to 12 days. The initial dose was usually 0.5 cc. with subsequent increase in dosage to as high as 5 cc.

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The usual procedure was to inject from 0.5 to 3 cc. of the toxin intravenously and study the kidneys at autopsy. In a few cases one kidney was removed 6 days after a single injection of the toxin and the animal was killed 13 days later for comparison of the kidney lesions.

Control Experiment: Sterile peptone broth was injected intravenously into the ear veins of normal rabbits for control of our experiment. The time of the injections and the amount of broth corresponded to the injections of the toxin.

Blood Analysis: Chemical analyses were made on the blood of rabbits to determine the carbon dioxide combining power of the blood and the content of non-protein nitrogen, chlorides, glucose and uric acid. Two cc. of blood were usually taken before each injection of the toxin. It was impossible to obtain a sufficient quantity of blood each day for the above determinations. Ten to 15 cc. of blood were obtained from the ear veins 4 to 5 days before the toxin was given and a similar amount was taken from the heart a few minutes before death. Since non-protein nitrogen of the blood was the only element that showed any constant variation from the normal, the remaining constituents that were determined are not included in this report.

Autopsies: A complete autopsy was performed and the tissues were fixed in Zenker-formalin and formalin.

Stains: Sections of the kidneys were stained routinely with hematoxylin and eosin and certain sections were stained for connective tissue by Mallory's aniline blue method and with scharlach R for fat.

Experimental

Eighty rabbits were given injections of staphylococcal toxin and of this group 58 animals died, and the other 22 rabbits were killed. Eighteen rabbits received intravenous injections of sterile peptone broth without a single death. The amount of toxin given and the length of time elapsing before death varied in the groups of rabbits. Some of the animals died immediately after the intravenous injection of 0.5 cc. of the toxin, while other rabbits received as much as 22.5 cc. over a period of 9 days before death occurred. The animals that received an initial dose of 2 to 3 cc. of the toxin usually died in 3 to 5 hours. Three rabbits that were given 0.5 cc. of the toxin and operated upon 6 days later showed only microscopic lesions in the kidneys, which will be described below.

The acute lesions produced in the kidneys varied in extent; however, the general process was always the same. The lesions may be divided into three groups.

In Group 1 are those rabbits that died within 12 hours after the first injection of the toxin. The kidneys of these animals were normal in the gross. In the microscopic sections the glomerular tufts are swollen and the capillary loops are dilated and filled with red

blood cells (Figs. 1 and 2). In some of the glomeruli there is an accumulation of an albuminous material in the space between the tuft and the capsule. The epithelial cells lining the tubules are swollen, especially in the convoluted tubules and in the loop of Henle. These swollen cells not only diminish the size of the lumens of the tubules but often occlude them. In the lumens of some of the tubules there is some albumin.

In Group 2 the kidneys in the gross were swollen and purplish red in color; on the cortical surface small, irregular, yellow areas, which extended downward into the deeper layer of the cortex, could be seen. In the microscopic sections the epithelial cells lining the tubules are swollen and necrotic and often form epithelial casts. Many of the epithelial cells that remain are filled with large hyaline droplets. In addition to the desquamated epithelial cells in the tubules there are many hyaline casts, hyaline droplets and albumin. A few red blood cells are present in the lumens of some of the tubules. The endothelial cells are slightly swollen in the capillary loops of the tufts; however, the loops are not distended with red blood cells, but show a few hyaline thrombi. In the space between the tuft and the capsule in some glomeruli there are occasional large degenerated cells and a few red blood cells. Polymorphonuclear leukocytes and large mononuclear cells are present in some of the glomeruli and similar inflammatory cells are present in areas of the interstitial tissue. The yellow areas noted in the gross (Fig. 5) in the cortex of the kidneys have the appearance of infarcts, although only rarely are blood vessels occluded by thrombi. The glomeruli, tubules and interstitial tissues are necrotic and many polymorphonuclear leukocytes are present in some of the yellow zones (Fig. 6).

In Group 3 the kidneys resembled grossly those in the second group; however, the cortical surface here was yellowish brown in color and the necrosis was diffuse, involving the entire cortex. When the kidneys were sectioned there was a definite line of demarcation between the necrotic cortex and the better preserved medulla. The histological changes in the kidneys in this group are essentially the same as described in Group 2; however, the process is more diffuse.

In the group that received a single injection of 0.5 cc. the kidneys removed 6 days later were grossly normal; however, microscopically they show focal areas in which the epithelial cells lining the tubules have undergone degeneration (Fig. 3). That these lesions are of a

transient nature is indicated by the fact that examination of the remaining kidney, removed at autopsy 13 days later, shows no abnormalities (Fig. 4).

The kidneys in Rabbit 1, which was killed on the 11th day after the first injection, were moderately swollen and the cortex was yellowish gray in color. Microscopically the tubules are filled with hyaline casts and desquamated tubular epithelial cells (Figs. 7 and 8). The non-protein nitrogen was 120 mg. per cent at the time of death. Many epithelial cells are absent along the wall of the tubules but the remaining epithelial cells are not swollen, as was noted in the kidneys in those rabbits dying in the acute stage.

Rabbit 3 was killed on the 19th day after the first injection of the toxin. The kidneys in the gross had small hemorrhagic areas and small gray areas on the cortical surface. Histologically a few of the glomeruli are found to have blood in the space between the tuft and the capsule.

A chemical analysis of the blood was made on a series of animals that received the staphylococcal toxin (Table I) and also upon the control group that received only sterile peptone broth (Table II), in order to determine whether there was any relation between nitrogen retention in the blood and the extent of damage in the kidneys. The majority of the rabbits receiving injections of the toxin showed a gradual elevation of the non-protein nitrogen until death, whereas the control group showed neither nitrogen retention nor histological evidence of renal damage.

The rabbits that lived for a period of time after the intravenous injection of toxin grew progressively weaker during the last few hours of life and some of the animals had convulsions during this period. The rabbits presented the same clinical symptoms as those described by Weld and Gunther[3] and Burnet.[6] A majority of the rabbits that showed necrosis of the kidneys had a decrease or a complete absence of urine in the bladder at the time of autopsy. From this observation it would seem that there occurred a partial or complete suppression of urine.

The amount of toxin necessary to produce death and the length of time elapsing before death were variable factors. Because of this variation a standard dose of toxin could not be determined. Frequently death occurred within the first 24 hours after the intravenous injection of only 0.5 cc. of toxin.

We were unable to produce chronic renal lesions, for the animals that received a sufficient quantity of toxin to produce any degree of necrosis in the kidney died within a few days.

TABLE I

Summary of the Results in 4 Rabbits that are Selected to Illustrate the Changes in the Non-Protein Nitrogen of the Blood After the Intravenous Injection of the Staphylococcal Toxin (Normal Non-Protein Nitrogen 30–45 Mg.).

Rabbit 1				Rabbit 3			
Day	Time	Toxin	Non-protein nitrogen	Day	Time	Toxin	Non-protein nitrogen
		cc.	*mg. per cent*			*cc.*	*mg. per cent*
1	3 P.M.	..	44	1	4 P.M.	..	43.4
4	2 P.M.	0.5	..	2	2 P.M.	0.5	..
5	2 P.M.	..	49	3	9 A.M.	..	60
5	2.30 P.M.	0.5	..	3	11.30 A.M.	0.5	..
6	2 P.M.	1	..	4	10.30 A.M.	..	71
7	10 A.M.	..	48	4	12 NOON	0.5	..
7	11 A.M.	1.5	..	5	9 A.M.	..	91
8	10 A.M.	2	..	7	9 A.M.	..	48.4
9	9 A.M.	3	..	18	1.30 P.M.	..	44.3
10	9 A.M.	4	..	19	9.30 A.M.	..	39.8
11	9.30 A.M.	5	..	20	9.30 A.M.	..	38.4
12	11 A.M.	5	..	21	8.30 A.M.	..	38
14	2.30 P.M.	..	120	21	1.30 P.M.	..	37.5
14	2.30 P.M.	Killed	..	21	1.30 P.M.	Killed	..
Both kidneys are moderately swollen and the cortex is yellowish gray in color. The tubules are filled with hyaline casts and desquamated epithelial cells.				The cortex of the kidneys shows small hemorrhagic areas and depressed gray areas. A few red blood cells are present in some of the glomerular spaces.			
Rabbit 2				Rabbit 4			
Day	Time	Toxin	Non-protein nitrogen	Day	Time	Toxin	Non-protein nitrogen
		cc.	*mg. per cent*			*cc.*	*mg. per cent*
1	11 A.M.	..	36.3	1	4 P.M.	..	39.7
4	2 P.M.	0.5	..	2	2 P.M.	0.5	..
5	9.30 A.M.	1	..	3	9 A.M.	..	46.4
5	5 P.M.	..	80.8	3	11.30 A.M.	1	..
6	8.30 A.M.	2	..	4	10.30 A.M.	..	96
6	9.30 A.M.	..	75.4	5	11 A.M.	..	112
6	9.30 A.M.	..	Died	5	11 A.M.	..	Died
Both kidneys show cortical necroses.				Both kidneys show cortical necroses.			

TABLE II

Summary of the Results in 3 Rabbits that are Selected from the Group Receiving the Sterile Peptone Broth. The Non-Protein Nitrogen of the Blood is always Within Normal Limits

Rabbit 5				Rabbit 6				Rabbit 7			
Day	Time	Broth	Non-protein nitrogen	Day	Time	Broth	Non-protein nitrogen	Day	Time	Broth	Non-protein nitrogen
		cc.	*mg. per cent*			*cc.*	*mg. per cent*			*cc.*	*mg. per cent*
1	4 P.M.	..	39.5	1	4 P.M.	..	31.2	1	4 P.M.	..	48
4	12 NOON	1	..	4	12 NOON	1	..	4	12 NOON	1	..
5	10.30 A.M.	..	39.6	5	10.30 A.M.	..	40.2	5	10.30 A.M.	..	42.6
5	12 NOON	1.5	..	5	12 NOON	1.5	..	5	12 NOON	1.5	..
6	10.30 A.M.	..	44	6	10.30 A.M.	..	39.6	6	10.30 A.M.	..	36.4
6	11.30 A.M.	2	..	6	11.30 A.M.	2	..	6	11.30 A.M.	2	..
7	8.30 A.M.	..	42.5	7	8.30 A.M.	..	36.7	7	8.30 A.M.	..	36.2
7	9.30 A.M.	3	..	7	9.30 A.M.	3	..	7	9.30 A.M.	3	..
8	8.30 A.M.	..	40.5	8	8.30 A.M.	..	35	8	8.30 A.M.	..	38.7
8	10 A.M.	4	..	8	10 A.M.	4	..	8	10 A.M.	4	..
9	2 P.M.	..	39.8	9	2 P.M.	..	34.5	9	2 P.M.	..	33.4
9	2.30 P.M.	5	..	9	2.30 P.M.	5	..	9	2.30 P.M.	5	..
10	8.30 A.M.	..	40	10	8.30 A.M.	..	35.8	10	8.30 A.M.	..	35.2
13	8.30 A.M.	..	39.5	16	1.30 P.M.	..	37	13	8.30 A.M.	..	38
14	8.30 A.M.	..	41.3	17	10 A.M.	..	38.8	14	8.30 A.M.	..	40.9
16	1.30 P.M.	..	38.2	17	10 A.M.	Killed	..	16	1.30 P.M.	..	41
17	10 A.M.	..	40					17	10 A.M.	..	35.7
17	10 A.M.	Killed	..					17	10 A.M.	Killed	..
The kidneys are normal both grossly and microscopically				The kidneys are normal both grossly and microscopically				The kidneys are normal both grossly and microscopically			

Discussion

Neisser and Levaditi [2] described in detail the character of the necrosis occurring in the rabbit's kidney after the intravenous injection of staphylococcal toxin, and Neisser and Wechsberg [7] state: "These changes can only be interpreted as infarcts which are sufficiently explained by the finding of thrombosed vessels, which as the microscopic picture shows, are caused by the rich disintegration of leukocytes which in turn we must look upon as a consequence of the leukocidin effect."

Although there is a destruction of leukocytes in the circulating blood [1] we have been unable to demonstrate thrombi composed only of leukocytes and, furthermore, it is our opinion that the process of infarction will not account for all the changes produced in the kidney after the intravenous injection of the staphylococcal toxin.

It has been suggested that histamine is present in the staphylococcal toxin in a sufficient quantity to produce the necrosis in the kidney. Dolman [8] states: "Histamine has been shown to be present in the toxin in amount strictly comparable to that present in the original nutrient broth. Such amounts of histamine could not possibly be responsible for any of the characteristic features of the toxin. Moreover, if the histamine content of the toxin be destroyed by incubating it with histaminase, the original properties of the toxin remain unimpaired."

From our study it would seem that the toxin injures the epithelial and endothelial cells of the glomerular tufts and the epithelial cells of the convoluted tubules and of the loops of Henle. Any degree of injury may be found from simple cloudy swelling to complete cellular disintegration. We cannot be sure what portion of the tubule is injured to the greatest extent; however, in some of the microscopic sections it is thought that the convoluted portion of the tubule shows the greatest destruction. In the early stage the capillary loops of the tufts are dilated and filled with red blood cells. Later, the capillaries contain only a few red blood cells. In the early process the endothelial cells of the tufts are swollen and only a few of these cells are destroyed. It would seem from this observation that the endothelial cells are more resistant to the action of the toxin than are the epithelial cells that line the tubules. A few of the glomeruli are severely injured by the toxin, as shown by the presence of

desquamated cells, leukocytes and red blood cells in the capsular space, and also by the presence of adhesions between the tuft and the capsule (Figs. 9 and 10) and thrombi in a few capillary loops.

All of the rabbits that died showed an elevation in the non-protein nitrogen; however, there is a wide variation in the total amount of nitrogenous products retained in the blood at the time of death. The retention of the non-protein nitrogen in the blood does not exactly parallel the extent of the renal lesion in all the rabbits. Those animals in which extensive kidney lesions are found show a significant elevation in the non-protein nitrogen and whenever extensive lesions are absent the non-protein nitrogen is within normal limits.

It is of interest to note the relation between the uric acid content of the blood and the retention of non-protein nitrogen. When the non-protein nitrogen increases to 80 to 90 mg. per cent the uric acid does not show any elevation. The normal non-protein nitrogen content of the blood is 30 to 45 mg. per cent and the normal uric acid level is 1 to 1.8 mg. per cent in this group of rabbits.

The lesions produced in the kidney after the intravenous injection of this toxin cannot be considered a true nephrosis, as defined by Fahr, on account of the damage to the glomerulus and of the retention of nitrogenous products in the blood.

Summary

1. A filtrable toxin from a hemolytic *Staphylococcus aureus* produces damages to the tubular epithelium and the glomerulus when injected intravenously into normal rabbits.

2. The most conspicuous lesion occurs in the tubules.

3. The glomerulus is damaged, as shown by the presence of albumin, desquamated cells and red blood cells in the capsular spaces, by the presence of adhesions between the tuft and capsule of other glomeruli, and hyaline thrombi in a few capillary loops.

4. There is a retention of nitrogenous products in the blood of rabbits receiving staphylococcal toxin intravenously.

5. There is no retention of nitrogenous products and the kidneys are normal in control rabbits that receive intravenous injections of sterile peptone broth.

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DESCRIPTION OF PLATES

PLATE 108

FIG. 1. The capillary loops of the glomeruli are dilated and filled with red blood cells. This rabbit received 0.1 cc. of the staphylococcal toxin subcutaneously and 24 hours later 0.5 cc. intravenously. Death occurred 4 hours after the second injection. Hematoxylin and eosin stain. × 200.

FIG. 2. Same as Fig. 1. Hematoxylin and eosin stain. × 550.

FIG. 3. The left kidney in this rabbit was removed 6 days after the intravenous injection of 0.5 cc. of the toxin. The necrosis of the tubular epithelium is focal in distribution. Hematoxylin and eosin stain. × 250.

FIG. 4. The right kidney of a rabbit receiving intravenously 0.5 cc. of the toxin (the left kidney is shown in Fig. 3). This animal was killed 19 days after receiving the toxin. There are no focal areas of tubular necrosis, as found in the opposite kidney. The débris in the lumen of the tubules is often found in normal rabbits. Hematoxylin and eosin stain. × 250.

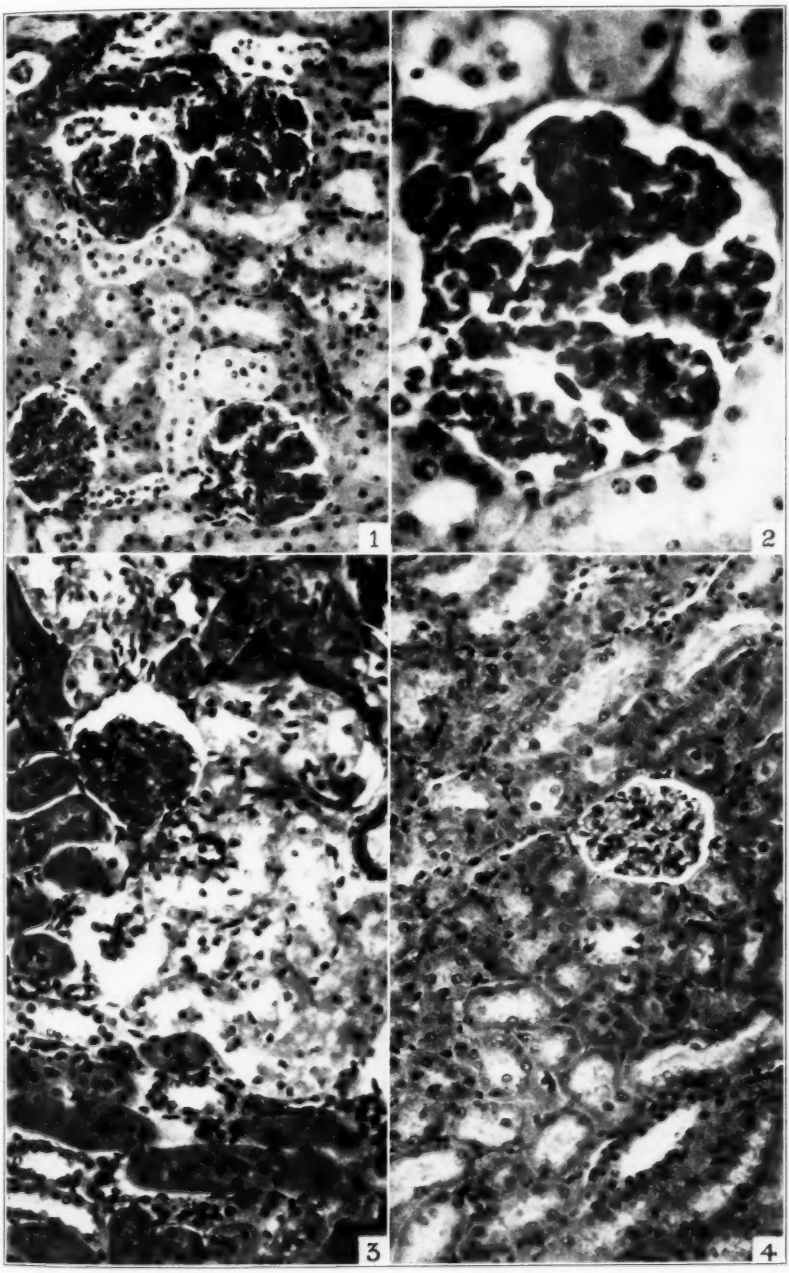
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PLATE 109

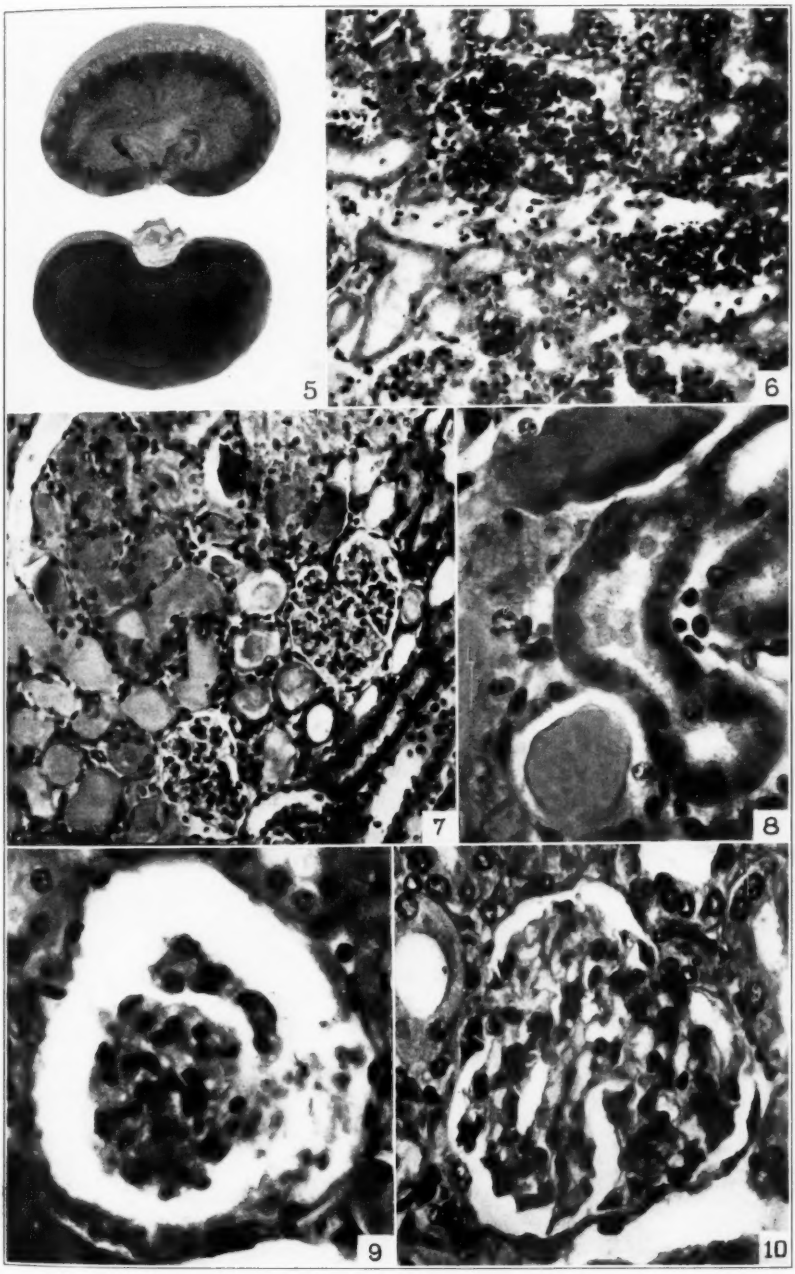
FIG. 5. Cortical necrosis and focal hemorrhagic areas in the kidney of a rabbit that received intravenously 0.5 cc. of the toxin and 24 hours later 0.75 cc. of the toxin. Death occurred 37 hours after the second injection.

FIG. 6. Rabbit 2, (see Table I). The tubular epithelium and the glomeruli are necrotic and polymorphonuclear leukocytes are infiltrating the interstitial tissue of this zone. Hematoxylin and eosin stain. × 250.

FIG. 7. Rabbit 1, (see Table I). The tubular epithelium is often completely destroyed and the lumens of the tubules are filled with hyaline casts. Hematoxylin and eosin stain. × 200.

FIG. 8. Same as Fig. 7. Hyaline droplets are present in the epithelial cells and hyaline casts fill some of the tubules. Many of the epithelial cells show these hyaline droplets in the kidneys which have been severely damaged by the toxin. Hematoxylin and eosin stain. × 550.

FIGS. 9 and 10. Rabbit 3, (see Table I). A few of the glomeruli have red blood cells in the capsular spaces. Other glomeruli show adhesions between the tuft and Bowman's capsule. Hematoxylin and eosin stain. × 550.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A HISTOLOGICAL STUDY OF THE ADRENAL CORTEX IN MONGOLISM *

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The etiology and pathogenesis of mongolism are still unknown. Since the condition was described by Down [1] in 1866 numerous hypotheses have been offered, and among these there may be mentioned syphilis, age of the parents, number of the pregnancy in mother's child-bearing history, alcoholism of either parent and exhaustion of the germ plasm. None of these has successfully withstood critical examination. A discussion of the most recent literature and theoretical considerations is given by Eley,[2] who concludes that the etiology of mongolism is still obscure.

The latest conjectures, as would be expected, have come from the field of endocrinology, with prominent mention given the pituitary, thyroid, and more recently the adrenal cortex. Because of certain evidence to be discussed it was thought that a study of the adrenal cortex by histological methods might yield data that would be of aid in evaluating the rôle of the adrenal cortex in the production of mongolism. We are not unmindful of the complex and still confused interrelations of the various parts of the endocrine system, nor do we forget the limitations of an examination of merely one organ in the study of a disease of obscure etiology. The purpose of this paper is to determine if there is any histological evidence in support of the theory that the adrenal cortex is in any way involved in the etiology and pathogenesis of mongolism.

THEORETICAL CONSIDERATIONS

Mongolism is as definite a clinical entity as cretinism. Definite stigmata are associated with the disease and a diagnosis can be made almost always at birth. That no basic similarity to cretinism exists has been shown by Talbot,[3] who proved by studies of the

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basal metabolism of mongols that "the thyroid is not involved in the majority of cases, and then only to a minor degree."

The task of relating any anatomical finding in the adrenal cortex to a hormone produced by the cortex will be a difficult one, for it is probable that the adrenal cortex produces more than one hormone. The work of Hartman and co-workers,[4] and Swingle and Pfiffner [5] on the hormone cortin, which prevents death from acute adrenal insufficiency, has been accorded general recognition. There is no suggestion of acute adrenal insufficiency in mongolism. If there is a lack in the adrenal cortex there must exist either a sublethal insufficiency, or insufficiency of a non-vital function of the gland. This latter consideration is probable in view of the possible multiplicity of adrenal cortex hormones.

A discussion of the relation of the adrenal cortex to the development of the central nervous system yields suggestions of value. A lack of cerebral development is an obvious feature of mongolism. That the adrenals may be absent or extremely hypoplastic in anencephalic or hemicephalic monsters has been well known since the first observation in 1842 by Johann Friedrich Meckel. Seventeen instances of this finding were reported by Lomer.[6] Weigert [7] and Zander [8] emphasized that the cortex of the adrenal was primarily affected in anencephaly.

Diffuse amyelination of the cerebrum has been described by Davidoff [9] in mongols. This finding is of interest in regard to the possible rôle played by the adrenals in myelination of the nervous system. Evidence for this has been adduced from the large size of the adrenal gland during the latter part of gestation at the time when cerebral development is said to proceed most rapidly, and the large amounts of cholesterol in the adrenal cortex during the first year of life, when the greatest activity in the myelination of the central nervous system is noted.

The marked lack of sexual development has long been observed in mongols, particularly in the males, and suggests the possibility of an adrenal cortex insufficiency. Though the relation of the adrenal cortex to sexual development is still a question, there are numerous examples of "adrenal virilism" with precocious sexual maturity and masculinization of females associated with tumors of the adrenal cortex.

A common clinical observation in mongols is the marked hyper-

flexibility and decreased muscle tonus. The decreased muscle tonus in Addison's disease, which is of unquestionable adrenal etiology, is a well known part of that disease picture. In addition, in common with patients suffering from Addison's disease, mongols tend to have low blood pressure and vagotonia.

Although no definite proof has been offered of any direct relation between the adrenal cortex and mongolism, these theoretical considerations do form a fair basis for the assumption that a hypofunction of the adrenal cortex is in some way related to the development of the condition known as mongolism, and justify an attempt by morphological means to test that assumption.

Materials and Methods

These studies are entirely morphological in character and are based on observations and measurements of a series of microscopic preparations from the adrenal glands of mongols. A series of 15 cases was assembled from the postmortem files of the Children's Hospital of Boston and all available data relating to the patients were studied. The diagnosis of mongolism was taken from the clinical diagnosis and only unquestionable instances were included in this series. All microscropic preparations were obtained from Zenker-fixed material and were stained by hematoxylin and eosin.

The essential procedure in this study has been the measurement of the width of the permanent adrenal cortex, as seen in cross-section. Measurements of the fetal cortex were also taken. These measurements were made with a micrometer ocular calibrated against a slide with a measured scale. Certain criteria of a true cross-section (*e.g.* cut in a plane perpendicular to the surface of the gland) were applied in an effort to obtain comparable data. Measurements were made only of those portions of the cortex in which the two opposite layers were approximately parallel to each other. For that reason the apex of a leaflet of the gland was not chosen (Fig. 1). Furthermore, only such places were measured where there was a full length longitudinal section of the fasciculata, which showed termination of the columns in a solid line rather than in a series of uneven blocks, as is found in sections that have been cut obliquely (Fig. 2). It was also endeavored to select areas for measurement where the two opposite layers of cortex were approximately the same width (Fig. 1).

Measurements were also made on a series of suitable controls, as will be indicated below. In the evaluation of these measurements some difficulty was encountered in arranging the series in order of relative maturity of the patients at the time of death. Because mongols lag behind normal children in development and because it is impossible to rule out the factor of prematurity, which would have an effect upon the actual age of the individual, it was decided to arrange the findings in two ways — by body length and by chronological age. It was not possible to obtain enough material for controls to match the mongol series by cause of death and sex, as well as age and body length, but these factors were taken into account wherever possible.

In most cases ten measurements were taken along the cross-section of the permanent cortexes satisfying the above criteria. If, however, the deviation of any one measurement from the mean of the ten measurements was more than one-third of the mean, a second set of ten measurements was taken. If the mean of the second ten differed from the mean of the first ten by more than one-third of the mean of the two sets the slide was rejected as being unsuitable for measurement. Consequently, the means recorded may be considered as statistically reliable measurements of the widths of the respective permanent cortexes within the limitations inherent in the method itself. Whenever the difference between the mean of the second ten measurements and the mean of the first ten was found to be within one-third of the mean of the two, the slide was retained and the probable error was calculated on a basis of twenty measurements representing the sum of the two sets.

Whenever possible the width of the fetal cortex was measured, as well as that of the permanent cortex, and the measurements were accorded the same statistical treatment as those of the permanent cortex. In many sections the fetal cortex was represented by scattered groups of cells, making measurement of its width impossible.

The measurements recorded in the tables were all made on fixed material. As no allowance was made for shrinkage by fixation, they do not represent a measurement of the absolute width of the cortex, but are probably somewhat less than the actual width in the fresh gland.

After arranging the means of the widths of the permanent cortexes of the mongols and the controls in body length and chrono-

logical age groups the arithmetic mean of each group was obtained for the purpose of comparing the means of widths of the permanent cortexes of the mongol groups with those of the respective control groups. These means will be found recorded in tabular form below.

TABLE I

Means of Body Length Groups

Mongols			Controls		
Group	No. cases	Mean of permanent cortex	Group	No. cases	Mean of permanent cortex
1 46–47 cm.	4	0.355	45–48 cm.	16	0.35
2 51–52 cm.	2	0.38	50–53 cm.	8	0.45
3 58–65 cm.	6	0.43	57–66 cm.	24	0.47
4 68–70 cm.	2	0.42	67–73 cm.	8	0.52
5 about 80–85 cm. ...	1	0.47	75–90 cm.	4	0.60

TABLE II

Means of Age Groups

Mongols			Controls		
Age groups	No. cases	Mean permanent cortex	Age groups	No. cases	Mean permanent cortex
1 7 mos. prenatal ... 2½ mos. postnatal to full term 3 wks.	5	0.36	7½ mos. prenatal.... 15 hours to full term 1 month	20	0.375
2 4½ mos. to 7 mos. .	5	0.40	4 mos. to 7 mos.	12	0. 46
3 9 mos. to 11 mos. .	4	0.45	8 mos. to 12 mos. ...	10	0.525
4 2 to 4/12 yrs.....	1	0.47	2 yrs. to 2 8/12 yrs. ..	4	0.605

RESULTS OF PRESENT STUDY AND DISCUSSION

A microscopic study of the adrenal glands of mongols reveals no constant pathological findings whereby a diagnosis of mongolism can be made in this manner. Our tables of measurements of the adrenal cortex show that the amount of variation among the members of various groups of the same age or body length is considerable. However, when the means obtained from the mongol group are compared with the means of the control groups it can be seen that:

1. The width of the permanent cortex of mongols in the first year of life does not differ essentially from that of controls of the same age.
2. There is a definite lag in the growth of the permanent cortex in the older mongols, as compared with the control group.

The magnitude of the difference is indicated in the tables. It can be shown from our study that there are individual cases in which the permanent cortex of the mongols is actually greater than some of the individuals of the same body length or age in the control group. The lack of a constant difference between the measurement of the permanent cortex of any given mongol and the control is a finding of great importance in evaluating the results of the present study. Since in the series the permanent cortex of several mongols was actually equivalent to, or greater than, members of the control group, the primacy of the adrenal cortex in the etiology of mongolism is definitely precluded. The difference in the means as maturity advances is, however, also an important finding in giving an indication that a tendency toward hypoplasia of the adrenal cortex is a part of the pathological picture of mongols. That this hypoplasia is real and not to be explained by the fact that mongols lag behind normal children in physical development is shown by the similarity of result by both the age and body length grouping. The body length arrangement controls the factor of physical retardation.

Speculation about the cause of the tendency toward hypoplasia of the permanent cortex of the adrenal gland in mongols is entirely beyond the scope of this paper. Although the primacy of the adrenal gland in the etiology of mongols appears to be ruled out by this study the hypoplasia of the adrenal cortex, though secondary to some primary cause, may well explain certain less essential manifestations of mongolism (the hyperflexibility and the lack of sexual development). If, as has been suggested by Macklin,[10] mongolism is hereditary and is due to the coming together of several recessive characters, the hypoplastic adrenal cortex may be one of the factors involved in a pleuriglandular endocrine insufficiency explained on such a genetic basis.

Summary

1. A method for measuring comparable widths of the adrenal cortex is described.

2. The lack of any characteristic pathological picture in the adrenal cortex in mongolism is noted.

3. The results of a measurement of the adrenal cortexes in a series of 15 mongols compared with 60 controls are tabulated and arranged in chronological age and body length groups. According to our measurements:

(*a*) There is considerable individual variation in the measured widths of permanent and fetal cortexes among the members of groups of similar age or body length of both the mongols and the controls.

(*b*) There are individual cases in which the permanent cortex of a mongol is actually greater than some individual controls in the same age or body length group.

(*c*) There is a definite retardation in the development of the width of the permanent cortex of the mongol adrenals so that there is an actual hypoplasia of adrenal cortex of older mongols, as compared with the controls.

Conclusions

As maturity advances, a definite hypoplasia of the adrenal cortex in mongols becomes evident by the use of histological methods in the measurement of the width of the permanent cortex of the adrenal gland.

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DESCRIPTION OF PLATE

PLATE 110

FIG. 1. Photomicrograph showing typical cross-section of apex of leaflet of adrenal gland, satisfying criteria of true cross-section; *i.e.*, full length longitudinal section of fasciculata terminating in solid columns (see text).

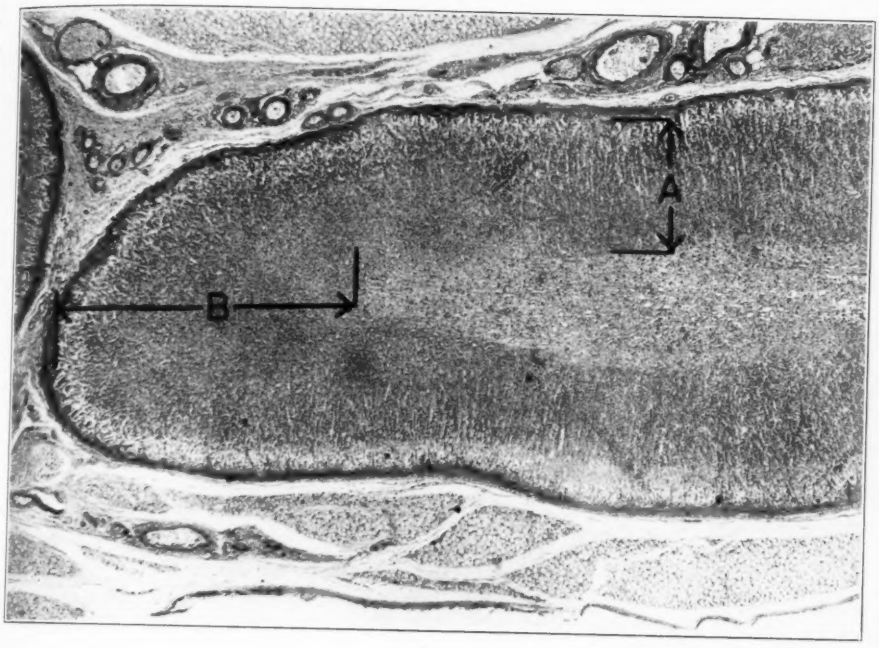
A = typical site of measurement for width of cortex with opposite layers of cortex approximately parallel.

B = width through apex not used as site of measurement. Hematoxylin-eosin stain. × 30.

FIG. 2. Photomicrograph showing example of oblique and coronal section of the type that was rejected as being unsuited for measurement.

A = oblique section of cortex.

B = coronal section of cortex. Hematoxylin-eosin stain. × 30.



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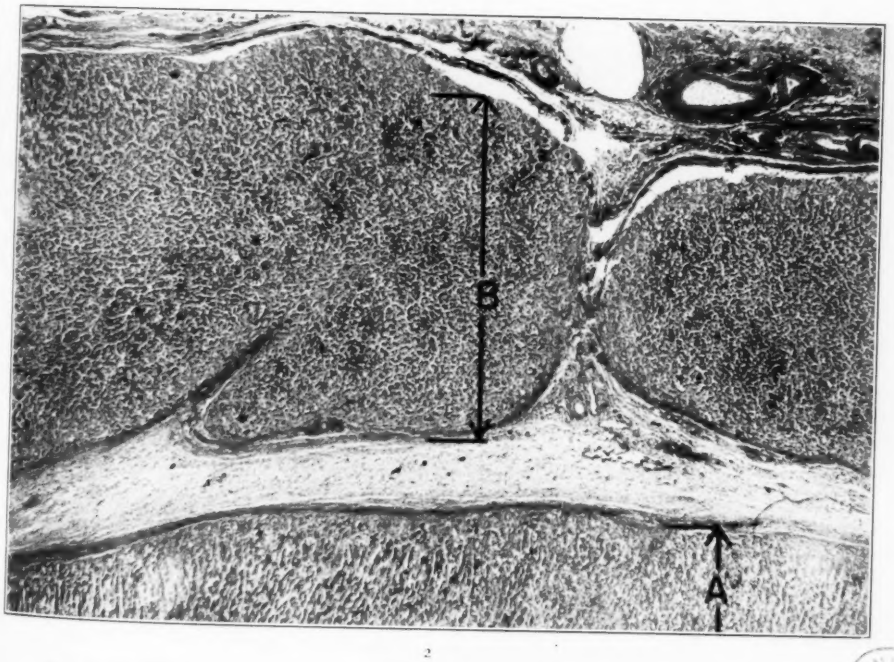

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Hirning and Farber

Study of Adrenal Cortex in Mongolism